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Biofertilizer use for Agricultural Sustainability

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Acknowledgements

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Biofertilizer Use for Agricultural Sustainability

Anwar Naseem

Abstract

In view of the economic and environmental considerations, it is imperative to explore alternatives to agrochemicals. This paper focuses on one use of nitrogenous fertilizers, and seeks out why alternatives are needed, and discusses the techniques of biofertilizer as a natural and feasible alternative to excessive use of nitrogenous fertilizers. It first discusses the benefits brought about by the use of nitrogen fertilizers to agricultural production in the world at large and Pakistan in particular. Constraints in making the use of nitrogen fertilizers more widespread because of costs and other consideration are also discussed. It then suggests the use of biofertilizer as a supplement to the use of chemical fertilizer in some crops and certain geographical areas. The paper describes the biofertilizer in some detail and the efforts made in applying this technology in Pakistan. It also explores the constraints on the use of biofertilizer in Pakistan and how to alleviate these through fuller research and policy measures. Some concluding remarks and policy recommendations are made in the end.

Introduction

Pakistan's economy is by and large an agricultural one. Not only does it provide for a growing food demand fuelled by a rising population, this sector of the economy is also the major force in the growth and development of the national economy. Roughly one-half of the gross domestic product is produced by farming and agribusiness, employing two-third of the population and accounting for three-fourths of the exports (Mahmood and Walters 1990). Such a heavy dependence on agriculture is symptomatic of Pakistan's underdevelopment. It is important therefore not only to reduce this excessive dependence but also to preserve the agricultural resource base through increased efficiency and higher productivity.

The increased productivity in agriculture should moreover be sustainable, that is the current resources must not be depleted, damaged, or affected in any other way that it shifts the problem into the future. Unfortunately, agriculture in Pakistan is neither efficient nor sustainable. Intensive irrigation, though responsible for substantially increasing agricultural output during the green revolution of the 60's and 70's, has also unfortunately led to water logging, salinity and sodicity. It is estimated that 1.5 and 5.5 million hectares are waterlogged and saline, respectively (Sheikh 1991). The negative effects of heavy fertilizer and pesticide, notwithstanding their low average level of application, are also now coming to light. Studies show that the quality of water in Sumundri in the province Punjab is deteriorating, and may be unfit for irrigation and drinking, because of increase in salinity and sodicity (Ali and Jabbar 1991). Moreover, the economic cost associated with agrochemical usage is considerable and cannot be overlooked.

Benefits and Constraints of Nitrogenous Fertilizers

Nitrogenous (N) fertilizers have had an important role in increasing food production commensurate with population growth during the last few decades, and are now considered an indispensable part of modern agricultural practices. Since the 1960's, the world consumption of N fertilizer has increased dramatically. Table 1 and Figure 1 shows that in 1960 the world consumption of N fertilizer was 9.2 million tons (Mt) which in 30 years increased to 80.3 mt, a 9 fold increase (Sheldrick 1990). The increase in consumption of N fertilizer resulted in an increase in crop yield. This was accompanied by an increase in the use of high yielding varieties of wheat, during the green revolution. It has been estimated (Russel et al. 1989) that in 1985, the use of 38.8 mt of N fertilizer on cereals globally resulted in increased world cereal production of 938 mt, more than half of the total cereal production.

Table 1: World Nitrogen Fertilizer Consumption 1960-90

Year	Fertilizer Consumption (Million Tons N)	% Increase
59/60	9.2	
64/65	15.3	66.30
69/70	28.7	87.58
74/75	38.6	34.49
79/80	57.3	48.44
84/85	70.5	23.04
89/90	80.3	13.90

Source: Sheldrick, W.F. (1990)

Similarly, in Pakistan the consumption of N fertilizers has also seen an extraordinary increase. Table 2 shows that since 1970 the consumption of N fertilizer has increased from 251.5 thousand tons of N in 1970 to 1471.6 thousand tons N in 1990. Moreover, it is estimated (on the basis of projected food requirements and expected yield increase due to fertilizer application) that the requirement of fertilizer in the year 2000 would be nearly 3,300 thousand nutrient tons (Sandhu 1992). Half of the requirement would have to be imported, as the local production is not expected to increase more than 1,500 thousand nutrient tons.

Figure 1:

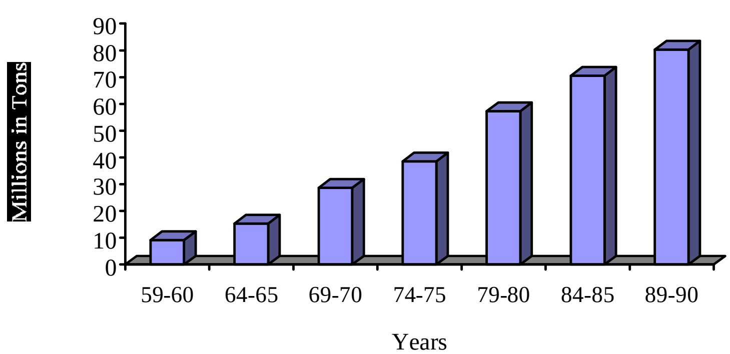


Table 2: Consumption of N fertilizer in Pakistan

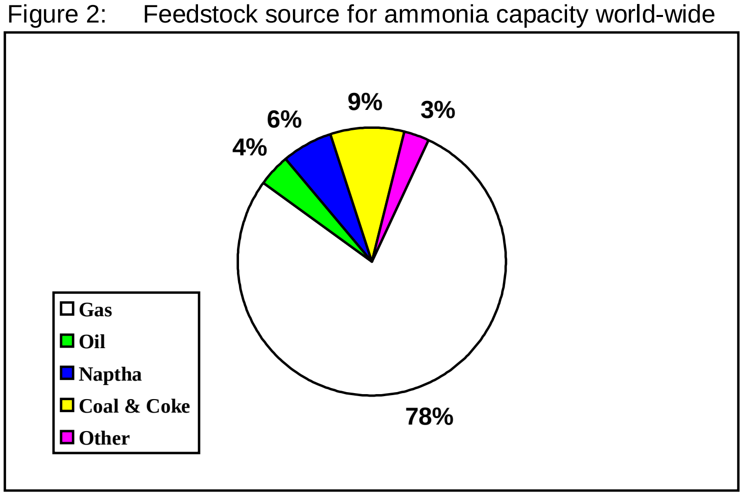
Year	Consumption in '000 N/tons
70/71	251.4
75/76	445.3
80/81	843.0
85/86	1128.2
90/91	1471.6

Source: Agricultural Statistics of Pakistan (1991-92)

There is no doubt about the gains made due to increased fertilizer usage on food production. However, in evaluating the benefits of N fertilizers, the 'costs' associated with its use cannot be ignored. These costs, both economic and environmental, must be considered if the process is to be sustainable. Modern agriculture is described as one being based on maximum output in the short term, with little regard for input efficiency or stock maintenance (Odum 1989): where the relevant stocks in agriculture are soil, water, non-renewable energy resources and environmental quality. Although nitrogen fertilizer ranks first among the external inputs for maximizing output, in the short term its long term efficiency is one of the lowest among plant nutrients, mainly because of its adverse environmental effects (Odum 1989).

Even from a purely economic point of view, nitrogenous fertilizers are very expensive to produce, their manufacture being highly-intensive in the use of fossil fuels. For example, the synthesis of ammonia from N2 gas (the Haber-Bosch process) requires large quantities of hydrogen usually obtained from natural gas (Fig 2), in addition to large amounts of energy to establish and maintain the conditions of high temperature and pressure required for nitrogen to react with hydrogen to produce ammonia. Add to this the energy utilized in transportation, storage, and application of N fertilizer, the cost increases furthermore. It is estimated (Bohlool et al. 1992) that 22,000 kilo calories of energy per kilogram of fertilizer N is processed, distributed and applied. Apart from production costs the capital cost for constructing a medium sized N fertilizer factory is estimated to exceed $100 million.[1]

Figure 2: Feedstock source for ammonia capacity world-wide



Source: Sheldrick (1990)

In Pakistan in 1989, 1113.77 Mt of N fertilizer was produced, of which 134.19 Mt was imported (Fertilizer Related Statistics 1989). Of the existing fertilizer plants in the country, it is estimated that 12.4 million cubic meters of gas is consumed, or about 27% of the total gas consumed in the country. Thus

1. It has been argued (e.g. by S. Marglin 1992) that the energy-intensity of green revolution agricultural inputs are highly exaggerated and that in relative terms they would account for no more than 8% of US energy consumption. While this may be a valid point to highlight the wasteful use of energy in the consumption patterns of developed countries, it does not detract from the necessity of conserving a non-reproducible natural resource through the use of alternative methods of augmenting agricultural production. Moreover, for an individual energy-deficit developing country, such as Pakistan it is imperative to look for indigenous sources of meeting energy demand in alternative uses.

fertilizer is a heavy consumer of natural gas. Further plants are expected to be commissioned to meet the growing demand costing millions of rupees. Moreover, as the production of N fertilizers is dependent on energy supply such as oil and gas, whose domestic prices tend to rise considerably over time, the costs of such fertilizers is generally rising. With such high and rising costs associated with the use N fertilizers alternatives, their is an obvious need for exploring low cost alternatives.

By far the most important set of arguments against the excessive use of chemical fertilisers are based on their direct and indirect environmental effects. The cost associated with environmental degradation and human health, far exceeds economic costs (Bohlool et al. 1992). In addition, there are certain other factors associated with the use of N fertilizers, with considerable adverse effects on the public health, especially of children. Nitrogenous fertilizers such as urea are converted into ammonia by common heterotrophic bacteria. Ammonia when oxidized by certain bacteria is converted into nitrite which is further oxidized into nitrate by Nitrobacter. Normally nitrite does not accumulate in soil, but is highly toxic to plants and micro organisms. Accumulation of nitrate in ground water, it has been suggested, is the cause of several human health problems, including birth defects, cancer, nervous system impairment, and methoglobinemia. Methoglobinemia is caused by alteration of some of the nitrate into nitrite into the bloodstream, and interaction of the nitrite with the haemoglobin in the blood to produce metheglobin, a form that does not carry oxygen to the body cells. Methoglobinemia is more common in infants as adults tolerate far higher concentrations than infants (Council for Agricultural Science and Technology 1985). Nitrates are also the precursors of nitrosamines and nitrosamides which are carcinogenic, mutagenic and teratogenic (Sandhu 1993).

It is therefore not surprising than that nitrate run off into surface water and aquifers is a major health concern. Although in Pakistan, a study (Ali and Jabbar 1991) has found that nitrate concentration is far below the toxic level, with the increase in the consumption of N fertilizers on the rise, the situation needs to be closely monitored. However, it has also been reported (Sandhu 1993) that the nitrate concentration in wheat flour ranged from 82.2-478.9 ppm, and extrapolated to estimate that about 104-239 milligrams of nitrate is being consumed daily by an adult (Sandhu 1993). In comparison, there is a maximum limit of 10 ppm nitrate in drinking water. Apart from endangering human health, the excessive use of N fertilizers also poses a threat to the environment. The gaseous oxides of nitrogen, derived from N fertilizers are highly reactive and are implicated in the depletion of the ozone layer. Table 3 gives the potential adverse effects of excessive N fertilizer usage.

Table 3: Potential negative effects of excessive fertilizer N application

Impact	Causative Agent
Human	
Methemoglobinemia in Infants	Excess NO_3 and NO_2 in waters and food
Cancer	Peroxyacyl nitrates, alkyl nitrates
Respiratory illness	NO_2, HNO_3, vapour in urban atmosphere
Environmental	
Environment	Excess NO_3 in feed and water
Eutrophication	Inorganic and organic N in surface water
Materials and Ecosystems damage	HNO_3 aerosols in rainfall
Plant toxicity	High levels of NO_2 in soils
Excessive plant growth	Excess available N
Stratospheric ozone Depletion	Nitrous oxide from nitrification, denitrification, stack emissions.

Source: Bohlool et al. (1992)

Keeping these issues in view, it becomes necessary to look at alternatives that will reduce the usage of N fertilizers. Alternatives which will not only maintain the current yields, but also have the potential of increasing them, while also being sustainable. The process of biological nitrogen fixation (BNF) offers one such alternative. However, BNF does not seek to eliminate the usage of N fertilizer, rather supplement it so that its consumption is decreased. Moreover, the application of BNF is not necessarily universal, that is not all plants are able to take advantage of this process, and those that do may be constrained by varying environmental conditions.

What is a Biofertilizer?[2]

Amino acids are the "building blocks of proteins", which are necessary for the normal function of life. Nitrogen (N) forms an essential component of amino acids, and is thus required by all living organisms. Elemental nitrogen is the most abundant of the gaseous elements in the atmosphere (78% by volume), however it is of little use to plants as it cannot be utilized in this form. Moreover, bound nitrogen which is utilized by plants is inadequate in our agricultural soils; after water, it is the most limiting factor in crop production.

Biological nitrogen fixation is a process whereby certain bacteria possessing the *nif* gene are able to convert (or fix) elemental N_2 into ammonia, which plants readily take up, and incorporate into complex biological compounds. According to recent estimates (Ishikuza 1992) biological nitrogen fixation adds more than four times (175×10^6 metric tons) as much nitrogen to the earth as all the synthetic nitrogen fertilizers of the world, which amounted to 44×10^6 metric tons in 1975. This figure demonstrates the significance of biological N_2 fixation in agricultural and natural N cycles.

The process of fixation can occur by associative and free living micro-organisms or through a symbiotic relationship between certain plants and micro organisms. Table 4 lists some of the nitrogen fixing organisms. Although the amounts of N_2 fixed by associative and free living micro-organisms is considered low, they are capable of contributing as much as 50% of N requirement of rice crop. It is when symbiotic N are exploited that BNF can be a major source of N in agriculture. The amount of N input is reported (Keyser 1992) to be as high as 360 kg N per ha.

Table 4: List of nitrogen fixing organisms

Free-living	**Blue-green Algae *Azobacter, Clostridium, Klebsiella***		
Associative	Plant	Associative organism	
	Grass	*Azobacter*	
	Sugar cane	azosprillium	
Symbiotic	Plant	Host genus	Microsymbiont
	Legume	Glycine	Bradyrhizobium
		Vigna	Rhizobium
	Non-Legume	Alnus, Myrica	Actinomycetes *'Frankia'*
	Lichens	Collema	Nostoc
	Waterfern	Azolla	Anabeana

Source: Ishikuza (1992)

The beneficiaries of Biological Nitrogen Fixation can be broadly grouped into three main ecosystems: namely rice paddies, tree ecosystems and upland crops (Peoples and Craswell 1992). However, from the point of view of biofertilizers, which has more commercial connotations, the benefits are limited to leguminous crops, especially in the Pakistani context. In fact, BNF and biofertilizers are often considered synonymous with

2. Following is a general overview of Biofertilizer and Biological Nitrogen Fixation. For a more thorough scientific and technical explanation of BNF the reader is refereed to Rao, N.S.S., *Biofertilizers in Agriculture.* New Delhi, India, Oxford and IBH publishing, 1982

legume-rhizobium symbiosis. Nevertheless, there are other systems such as Azolla-anabaena symbiosis which is an important source of nitrogen for rice, and in many countries this algae anabaena symbiosis is being used as a biofertilizer. However, in Pakistan biofertilizers intended for crops other than legumes are practically non-existent, although there are institutes doing research on such fertilizers.

Legume-Rhizobium symbiosis

Legume-rhizobium symbiosis is the best understood and quite well exploited system in which the legume derives most of its nitrogen from rhizobium, which forms colonies on the roots of the plants called nodules and is thus able to fix nitrogen. Leguminous crop are one of the largest families of the flowering plants with some 625 genera and 18,000 species, and are also one of the most important economically (Forbes and Watson 1992). Its species yield seeds that are rich in protein and constitute valuable sources of food and fodder. Examples include the peas and beans of the temperate climate, and soybean, cowpea, lentil and groundnut which are grown quite extensively in our region. Other legumes are valuable as pasture plants (e.g. clovers) or green manure (e.g. Lucerne).

In Pakistan legumes are grown on 2 million hectares of land or about 10% of the total 20.9 million land under utilization (Agricultural Statistics of Pakistan 1991-92). The main leguminous pulse crops are chickpeas, mungbeans, blackgrams, lentils and soybean while alfalfa, clover, sesbania, vetch, and ipil-ipil are widely grown as fodder and green manure crops. Area and production wise, Punjab is the major pulse growing province, followed by Sindh and North West Frontier Province. Although Balochistan does not grow much pulses it is regarded as having a great potential for the commodity (Malik et al. 1985). Table 5 gives the area, production and yield of major pulses in Pakistan.

Table 5: Area and production of major Leguminous crops in Pakistan

	75-76	80-81	84-85	90-91
Mung				
Area ('000 ha)	67.3	67.0	104.2	141.1
Production ('000 tonnes)	31.9	31.8	48.8	56.5
Mash				
Area ('000 ha)	58.4	68.2	88.8	79.1
Production ('000 tonnes)	29.8	33.9	38.8	36.8
Masoor (Lentil) [a]				
Area ('000 ha)	86.8	65.4	67.9	58.7
Production ('000 tonnes)	33.6	27.7	29.9	26.7
Soybean [a]				
Area (ha)	N/A	4906	4457	1875
Production (tonnes)		2086	1602	930
Chickpea [a]				
Area ('000 ha)	1068.4	1128.5	1013.7	1091.5
Production ('000 tonnes)	601.4	313.4	523.7	531.0
Guarseed				
Area ('000 ha)	334.5	262.0	395.7	336.6
Production ('000 tonnes)	257.8	165.0	253.2	263.9

Source: Agricultural Statistics of Pakistan 1991-92
Note: a: = The decline in land area/production was a result of the substitution in favour of traditional crops

Since legumes have a high nitrogen requirement, the benefits of employing BNF are enormous (Singleton 1989). The seed protein content of legumes such as mungbean, peanut, and cowpea range from 24-28% and soybean protein can be high as 45%. This is in contrast to most cereal crops whose nitrogen content is less than 14%. Thus to produce a ton of legume seed would require more than twice as much nitrogen than to produce cereal crops. As crops are unable to take up applied nitrogen fertilizer with 100% efficiency it would require an application from 120 to 240 kg of fertilizer N/ha (160 to 320 kg urea/ha) to produce even this low yield (Singleton 1989). Soybean production would require almost twice this amount. Almost all this nitrogen can be supplied via BNF. If BNF is not exploited than the farmer will either obtain low yields due to nitrogen deficiency, or incur substantially higher production costs by applying nitrogen fertilizer.

Though the role of nodule bacteria in fixing nitrogen symbolically has been known for less than a century, the benefits that result from cultivation of legumes have been realized for thousands of years. Crops producing food rich in protein, while needing little, if any, nitrogenous fertilizer, have obvious potential. Therefore it is no surprise that nodulated legumes became major forms of farming systems all over the world.

Among the legumes that are important in agriculture, 98% of species are estimated to be able to form root nodules and wild species are mostly nodulated by specific strains of rhizobia in their natural habitats (Forbes and Watson 1992). Continual natural growth, or cultivation of legume usually leads to a gradual accumulation of suitable rhizobia in the soil. Unlike root pathogens, these bacteria enter into a partnership, or symbiosis, with the host plant. Bacterial growth within the root is controlled by the legume, which provides energy to the bacteria for reducing atmospheric nitrogen gas to ammonia. Satisfactory crop yields often depend, especially when nitrogen compounds are deficient, on the establishment of effective (i.e. nitrogen-fixing) associations of the host plants with an appropriate strain of rhizobia, but the right strain does not always occur naturally where the crop is to be grown, in which case the desired bacteria should be introduced. Table 6 lists the six strains of Rhizobium and their host legumes.

Table 6: Rhizobium and their host legumes

Rhizobium Species	**Host Legumes**
Rhizobium trifolli	Berseem (*Trifolium alexandrinum*) Shaftal
Rhizobium leguminosarum	Lathyrus, Pisum, Vicia and Lens
Rhizobium phaseoli	Mong (*Phasolua aureas*)
Rhizobium meliloti	Senji (*Melillotus praviflora*)
Rhizobium japonicum	Soybean
Rhizobium lupini	Lupinus and Ornithopus

Source: Chaudhry, mimeo

Once the importance of legume-Rhizobium was realized, methods were devised to introduce rhizobia into the soil to benefit particular crops: the practice of soil and seed inoculation (or the usage of biofertilizer) had started. The capital cost of introducing inoculation technology is low, transportation costs are negligible and the techniques are simple enough for use in agricultural systems in the developing world.

Traditionally rhizobia have been introduced into the soil by the method of seed inoculation. The method is to place a large number of effective rhizobia, specific for that particular host, on a seed to increase the chance that the seedling will quickly become nodulated by the selected bacteria. However, rhizobia do not survive for long when tried on seed and the cultures themselves have a short life (Hamdi and Ruschel, mimeo). To overcome this, carrier based inoculant were developed. The purpose of a carrier is to maintain the viability of the cells, partly by protecting the bacteria against desiccation, to dilute them so that they will be distributed more evenly among the seeds being treated, and to help the bacteria adhere to the seeds. The carrier used almost universally is peat. Unfortunately, peat is unavailable in Pakistan, being only available in some areas of the North. However, alternative carriers have been evaluated for efficiency, and are discussed later on.

The major steps in the production of Rhizobium inoculants or biofertilizer involve cultivating the Rhizobium in a suitable medium, transferring it to a carrier such as peat, and finally packaging it. Depending on the size of inoculant required, the whole process can be very simple or quite complex. If a small amounts of inoculant is required it could be done at the lab level, that is cells could be cultured in a flask, transferred to a packet containing the carrier and subsequently sealed. If a larger amount of biofertilizer is desired, than such a process could be done with automation and using fermentors for culturing the bacteria. In both instances it is important that quality control is maintained at all levels, otherwise there is a danger of contamination of the final product. If contamination does occur, the inoculant will not be effective, and may even have harmful effects.

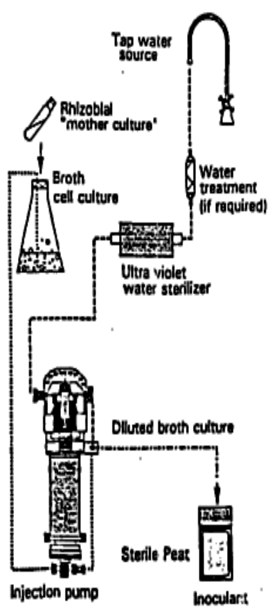
NifTAL, a center in Hawaii that does research in BNF, has developed an efficient and economical production unit for rhizobium (Fig 3), which is worth mentioning. This production unit is based on two key concepts: 1) the use of sterile inoculant carrier and 2) the dilution of broth cultures of rhizobia. This is in contrast to conventional production units which required large volumes of rhizobial broth mixed directly with non-sterile carrier before packaging. By using the dilution technique, the number and size of ferment vessels is reduced. By using sterile peat-based inoculants, the shelf life of inoculants is increased and less stringent storage conditions are required. Such a unit is cost-efficient, decentralized, and appropriate for small scale commercial inoculant production unit, which can be easily be adapted for countries like Pakistan.

Thus legume cultivation, aided by seed inoculation, can often improve an environment by promoting vegetation to combat desertification, assist the control of soil erosion, reduce wind movement of soil and help to reclaim degraded land. Most nodulated legumes are fully capable of meeting all their nitrogen requirements, provided of course, that other conditions, including availability of water and non-nitrogenous nutrients, are satisfactory.

Azolla-Anabaeana

Among the other sources of nitrogen fertilizer, the Azolla-Anabaena symbiosis is also quite important. Azolla is a free floating fern associated with the nitrogen fixing alga *Anabaena azollae*. Azolla plants form dense mats on water surfaces in ponds, ditches and rice fields throughout the warm, temperate and tropical regions of the world. Each leaf lobe has a cavity containing the alga *Anabaeana azollae*. By using Azolla, the nitrogen requirement for growing rice on flooded or irrigated land can be met. It has been estimated that this symbiosis can fix 100-170 kg N/hectare/year (Ali and Malik 1989). Azolla has long been used both as green manure for rice and as a fodder for poultry and livestock in South East Asia. Inoculation with Azolla results in a rapid growth, a suppression of weeds and a generous production of nitrogen. Yields of over 30t/ha of fresh weight are possible, containing over 2.5t/ha dry matter (Ali and Malik 1989). It is thus possible to use Azolla as a biofertilizer for rice paddies.

Figure 3: MPU Production Flow



Schematic of Micro Production Unit as developed by NifTAL

BNF Research in Pakistan

The work being done on BNF in Pakistan is extensive, but has been limited to basic lab research involving the efforts of isolated groups from the provincial and national agricultural research institutes. Among the more active and prominent institutes in this field today are the National Agricultural Research Center, Islamabad and the National Institute for Biotechnology and Genetic Engineering, Faisalabad (a brief historical and current research status of these institutes is given below). However over the years, several other universities and institutes did involve themselves in BNF, albeit for a short period. These groups worked independently of each other, as there was no national research plan on BNF. On the basis of their research and some further efforts a production of a few commercial rhizobium inocula (biofertilizer) has taken place.

Until recently commercial production of inocula was not available in Pakistan. For a short period the provincial research institute at Tarnab produced some inoculant, but this activity was discontinued when the key individual left. There have been other instances of such sporadic production of inocula, but these have been discontinued. The main reason for the discontinuation has been that there is a lack of manpower and funds resulting in poor quality inocula.

In 1980, plans were made to start a countrywide co-ordinated BNF Programme. Initially, this consisted only of a loose association of persons interested in BNF. Later, even though that a National Co-ordinator for the programme was appointed, the program never really got the funding in order for it to materialize. The program was revived again in 1984, this time with funding and a better organizational structure. Legume inoculation trials were to begin, whereby the need for inoculating leguminous crops would be determined. Such trials are conducted to give an indication as to which crops respond to inoculation. In the case where a response is not obtained, whether the yield obtained represents the maximal yield obtainable. If it does then there is no problem. If it does not then research is indicated to find strains of Rhizobium that will produce top yield. Much of the research that is done on BNF in universities and institutes is concentrated on ways of improving the yield through inoculation, given the constraints of the existing environmental conditions.

Several studies have revealed that inoculation can in fact increase yield of legumes in Pakistan. Qureshi et al. (1986) carried out inoculation studies with *Rhizobium japonicum* at different locations of the Peshawar valley to investigate the effect of such inoculation on soybean yields. The results revealed that the yields of un-inoculated soybeans were significantly lower than inoculated ones at all locations. Moreover, the height and number of pods were significantly lower than inoculated ones at all locations. Table 7 summarizes the results obtained from two locations.

Table 7: Results of inoculation trials on soybean

At Nawtnkili, Mardan in kharif 1983

Characteristic	Inoculated	Un-inoculated
Plant Height (cm)	75	62
Pods per plant	28	21
Plant population/ha	243,000	256,000
Seed Yield (kg/ha)	2126	1655

At Akbar Pura, Peshawar in spring 1985

Characteristic	Inoculated	Un-inoculated
Plant Height (cm)	68	62
Pods per plant	31	23
Plant population/ha	282,000	294,000
Seed Yield (kg/ha)	2137	1388

Source: Qureshi et al. (1986)

It was further calculated that the effect of inoculation of bean yields was statistically similar to fertilizer doze of 200 kg/ha nitrogen. This was done by treating the soybean with different amounts of nitrogenous fertilizer and comparing the results with that of inoculated and un-inoculated treatments. Table 8 gives a summary of the results.

Table 8: Results of inoculation and fertilizer treatment on soybean plant

Treatments	Plant/ha ('000)	Pods/ Plant	Beans/ pod	Plant/ height (cm)	Bean yield (kg/ha)
N-P_2O_5 (kg/ha)					
50-50	224	12.46	1.96	43.67	895
100-50	241	13.80	2.21	46.83	1095.68
150-50	281	15.76	2.21	49.67	1195.98
200-50	215	18.00	2.02	53.16	1290.13
Inoculum+ 50 kg P_2O_5 /ha	232	20.73	2.04	55.00	1364.20
No Inoculum+ 50 kg P_2O_5/ha		11.46	2.12	41.17	865.74

Source: Qureshi et al. (1986)

A similar study was done by Idris & Sandhu on the effects of inoculation on lentils. They inoculated seven different strains of Rhizobium; the results revealed that the dry matter yield increased by 13.9 to 15.1%, grain yield by 10.2 to 28.6%, and the nitrogen fixing efficiency also increased by 21.6 to 85.63%.

Both these studies reveal that inoculation can have a beneficial effect, by way of increased yield and nitrogen rich soil, on Pakistani agriculture. However, it should be remembered that inoculation does not always result in increased yield, as much is dependent on the soil nitrogen content at the time of inoculation. The above studies show a better yield because they were conducted on nitrogen poor soil. This is especially true for the Peshawar study, as soybean is not traditionally grown in that area and was introduced to a very nitrogen deficient soil.

Once the importance of inoculation became apparent much attention was then given to the large scale commercial production of inoculants. The major donor for the country BNF program was USAID, who felt that such a venture would be more appropriate for the private sector to handle. The role of government agencies would then have been to ensure quality control and carrying out research. Although the private sector does have the capacity to produce inoculants, as many drug firms and penicillin plants have fermentation capability, none are enthusiastic to invest, for a variety of reasons, mainly because they feel that the market has not been created for it to have a potential. It was then decided to establish a pilot plant, which when fully functional would be transferred to the private sector. The pilot plant was built in the premises of NARC in 1986 and has been functioning since 1987.

BNF at NARC[3]

The Biological Nitrogen Fixation program at NARC was initiated in 1981, as part of country-wide co-ordinated effort on BNF, with emphasis on research into legume rhizobium symbiosis. The primary objective of the program in its initial phase was to carry out a survey of nodulating legumes from various ecological regions of Pakistan, while at the same time isolating efficient indigenous strains of rhizobia. Such studies are necessary before any inoculant production can occur as it is important to identify the right strain for the particular leguminous crop.

On a global basis, it has been estimated that only 15% of the legumes have been examined for nodulation while the rest still await inspection (Forbes and Watson 1992). Pakistan's flora is rich in Leguminous crops; prior to 1980, the only survey on the nodulating ability of legumes was done by Athar and Mahmood (1978, 1980) who surveyed 91 species of which 15 were new findings. By 1992, the NARC team had acquired seed collections of various plant species from different international organizations which are known or likely to fix atmospheric nitrogen. Once such plants are introduced, they not only increase the diversity of plant species in the country, but if they are able to fix nitrogen also help in increasing the soil N content.

During the past decade, NARC has also collected 56 strains of rhizobia from various fodder, grain and wild legume species. This collection comprises of different rhizobial strains, accrued both locally and from abroad. All such strains are screened for their effectiveness for various crops, which helps in identifying strains which may be used in inoculant production.

Besides establishing the promiscuity of Legume rhizobia symbiosis, the NARC team also studied the ecophysiological constraints which affect the nodulation of legume crops under field conditions, such as salt stress. They have also carried a comparative study on the effectiveness of Rhizobium inoculation relative to that of mineral fertilizers. Like the Peshawar study, it was found that inoculation along with P and K gave comparable yield to nitrogen fertilization at both the fertility levels and increased significantly protein.

One of the major constraints for fuller implementation of biofertilizer technology relates to the non-availability of a suitable carrier. As mentioned, a carrier is the material in which the rhizobia are mixed, packaged, distributed and applied to the seed or the seed environment. The carrier that is regarded as being the most effective is peat. Since peat is unavailable in Pakistan researchers at NARC

3. This section draws from Annual Reports of PARC, and periodic reports of the BNF program

evaluated alternative carriers, such as bagasse, coal, composted tree leaves and soils containing certain levels of organic matter. It was found that indigenous mineral soil can also be used as a substitute carrier for peat (Khalil et al. 1991), and is now widely used by them.

One of the more notable accomplishments of the BNF program at NARC was the establishment of a pilot legume inoculant production unit, the first of its kind in the country. This unit consists of four functional areas namely 1) drying, grinding and storage of carrier materials, 2) fermentation area 3) a quality control lab and 4) an inoculant storage area. The whole unit in its present form has the capacity to produce 850 kg of inoculant in a 10 days cycle. The inocula are provided, on demand, for a variety of Leguminous crops such as soybean, mungbean and lentils. Initially the inoculant was supplied to various research organizations and projects for experimental and research purposes. Later it was provided to farmers, who have received it well as can be seen from the increase in demand. Table 9 shows the increase in demand for such inoculant over the years. It takes four bags to inoculate each acre, thus in 1991 approximately 20,000 acres were inoculated. However, the real figure may be much less as some areas are inoculated more than once during the year. Nevertheless the figure is less than a percent of the potential 5 million acres (of leguminous crops) that could be potentially inoculated.

Another area of BNF that was studied at NARC involved Azolla-anabaena symbiosis. Here basic research was carried out with the objective of using Azolla compost in rice as N-Biofertilizer. However since 1988, work on Azola-anabaena has been discontinued, and now only the Legume-rhizobium symbiosis aspect of BNF is studied.

Table 9: Supply of Rhizobium inoculants by NARC

Year	No. of Bags Supplied	Crop Inoculated
1987	463	Soybean
1988	1335	Oilseed
1989	1120	Soybean Mungbean Alfa Alfa
1990	4802	Oilseed
1991	5025	Soybean Chickpea

BNF at NIBGE[4]

The BNF program at the National Institute of Biotechnology and Genetic Engineering, Faisalabad (formerly at the Nuclear Institute of Agriculture and Biology) is more structured and organized. It is probably the only institute that has been able to sustain its research activities in BNF for the past 20 years. Over the years its research has evolved into four major areas namely: 1) Symbiotic nitrogen fixation (Legume-Rhizobium symbiosis), 2) Actinorrhizal symbiosis, 3) Associative nitrogen fixation and 4) Azolla-Anabaena symbiosis. For the purpose of this paper, the discussion will be limited to the former two, because these areas not only do they constitute the major research activities of the institute, but they also have a more realistic role in Pakistani agriculture.

Legume-rhizobium symbiosis has been a research area since the early years of the institute, and is now one of prominent aspects of the program. Some of the activities that have been done on Legume-Rhizobium symbiosis cover the following:

1. Culture collection of rhizobium/bradyrhizobium. Almost 210 local and exotic rhizobium have been preserved in a viable and genetically stable state.
2. Selecting and identifying superior strains of rhizobia and legumes for application in agriculture.

4. This section is based on the five year reports of NIAB

3. Evaluation of different carrier materials. It was found from this study that filter mud could be a suitable carrier and can be used as a substitute for peat.
4. Inoculation trials have been done for a variety of crops. These have included mungbean, lentil, chickpea and lentil. Once again the results show that the yield increase due to inoculation, and the increase is even more dramatic with the application of Phosphorus along with the inoculum.
5. A study on the competition between inoculated and indigenous Rhizobium/Bradyrhizobium. This study revealed that though inoculation may increase nodulation of the crop, yield may not necessarily increase along with it. Thus a low native rhizobial population is not the only criterion for success with inoculation as the host cultivator, soil type and environmental factors may also affect the establishment of active N_2 fixing symbiosis.
6. Several studies have been undertaken to evaluate nodulation under conditions of stress. These studies are useful as environmental factors such as salinity, high temperature and drought affect the nodulation and consequently nitrogen fixation capability. Strains that are able to survive such adverse conditions and at the same time efficiently fix nitrogen are highly desirable and thus selected for.
7. Production of Rhizobium inoculant. Rhizobium inoculum for soybean, chickpea, lentil, blackgrams and mungbean have been prepared. However, this is done at a lab scale, unlike the NARC program which has developed fermentors for inoculant production. This limits their inoculation capacity and inoculants are provided only on demand and not on a regular basis.

Several studies on Azolla have been conducted at this institute. The major ones are briefly described below:

1. One study was carried out to determine the effect of Azolla inoculation on rice yield using a variety of Basmati rice. The results revealed that upto 37% increase in grain yield is possible due to Azolla inoculation. This is equivalent to the addition of about 30 kg N/ha, thus a reduction in nitrogen fertilizer use is possible with Azolla inoculation in rice fields.
2. A problem with Azolla cultivation is that it is very susceptible to pests such as the aquatic snail and the water beetle larvae. In fact one of the reasons why work on Azolla was discontinued at NARC was because their nursery suffered a snail attack. The group at NIBGE has evaluated several pesticides of which "Furadan" was selected as being almost non-toxic to Azolla, but still capable of controlling the pests.
3. A survey of the major rice growing areas of Punjab was done to determine the natural distribution and ecology of Azolla. It was observed that Azolla grows only in the winter months (Nov-Apr), and is sensitive to high temperature, which prevails during the rice season, making its use difficult. Efforts to overcome this have resulted in the selection of a heat tolerant strain that is able to grow at temperatures prevailing in the Punjab.

Constraints to Utilization of Biological Nitrogen Fixing Systems[5]

Although there are still many scientific unknowns to completely understand the N_2 fixation, few, if any, are constraints in implementing the existing BNF technology. This is especially true in Pakistan's case where so much of the existing technology is not being used. Therefore, it is important to devote more efforts on adoption of what is already known. Nevertheless, there do exist obstacles for biofertilizer use. These are not only technical problems, but also as in Pakistan's case, socio-economic and human resource obstacles. The technical problems can be addressed through a comprehensive program of

5. This section is based on the article by Bohlool et al (1992).

basic and applied research, which, to a certain extent, has been done in Pakistan. Overcoming the socio-economic and human resource obstacles would require an emphasis on education, training and the promotion of private-enterprise development

Environmental Constraints

Of the technical obstacles faced, the soil-environment plays a major role. It is important to realize that the adverse factors of the soil environment may limit yield increase. Thus without a thorough understanding of the ecology of the various nitrogen-fixing systems, a successful application and acceptance of BNF technologies may not occur. As a rule of thumb, for inoculation to increase yield, there must be insufficient nitrogen available in the system to meet the crop requirement in the absence of inoculation. The major consideration affecting the microbe, the host or their symbiotic interaction includes soil acidity, other soil related factors including aluminium and manganese toxicity and calcium deficiency, phosphorous deficiency, salinity and flooding. Moreover a low yield potential of the legume crop due to soil management problems such as poor soil fertility, lack of sufficient water or severe insect damage limit the crop's requirement for nitrogen. Thus when management practices raise the yield potential of the crop (raise the crop nitrogen requirement) then the farmer will derive greater benefit to inoculation. Inoculant must be employed at the farm level with a package of other appropriate inputs in order for the farmer to derive full benefit from BNF.

There is also the problem of competition from native organisms in the soil which limit the successful establishment and effective performance of introducing N_2 fixing systems. This has been extensively demonstrated for legume inoculants, and is likely to be a problem for other exotic organisms in new environments. The complexities involved in competition for nodulation of legumes are numerous, and can be as varied as the production of antibiosis elements by native organisms to simply a better capability of nodulating. Nodulation in favour of the introduced Rhizobia can be achieved by altering environmental influences such as soil temperature, or additions of P and K. The population size of naturalized rhizobia has also been shown to affect the likelihood of inoculant establishment and the magnitude of a legume crop's response to applied rhizobia. Thus the more the rhizobia in the soil, the better the chances of nodulation, as it increases the competitive ability of the bacteria. This also has the advantage of inoculating less frequently, after a large population has been established in the soil.

Biological Constraints

There are also limitations imposed by certain biological factors which are more problematic to overcome but not necessarily impossible. The success of a BNF system, like any other biological process, is ultimately dependent upon the genetic potential of the organism and how that interacts with components of the environment. There are a number of biological factors that influence the expression of BNF in all nitrogen-fixing systems. In symbiotic associations, both partners are subject to biological constraints, like disease and predation which can directly or indirectly affect the amount of nitrogen fixed, as well as the quantity made available to other cropping system. In general, and especially so in legumes, the amount of nitrogen fixed is directly related to the growth potential of the host in a particular system. When growth is limited, for example by disease, nitrogen fixation will be reduced accordingly. Such biological factors can be alleviated by genetic manipulation or environmental manipulation or both, resulting in organisms which may be disease resistant, and more competitive.

Methodological Constraints

Methodological constraints also tend to limit proper utilization of biological nitrogen fixation. One such is the lack of reliable techniques for measuring nitrogen fixation in the field. As mentioned previously, it is necessary to quantify the amount of nitrogen available before the application of inoculants, to assess how successful it will be. Even after the introduction of bacteria into the soil, one needs to monitor the introduced rhizobia. Although this is relatively easy with symbiotic legumes, as this can be accomplished through serological means, it is a barrier for most other BNF systems. Studies have been initiated whereby rhizobium strains are differentiated on the basis of their sensitivity to various antibiotics. These studies also lead to isolation of antibiotic resistant strains of selected rhizobium strains.

Production Constraints

Inoculant technology is well-developed for legume inoculants, but still is in its infancy for actinorhizal and other BNF systems. Although there has been some success in growing Frankia in pure culture, it still remains a problem to produce inoculum on a large scale. Even for legumes, the scale of production, the availability of suitable carrier material, preservation of germplasm and shelf-life of the finished product are serious constraints to the use of inoculants, especially in Pakistan.

Socio-Economic Constraints

It is important to emphasize that the limitations to a fuller adoption of BNF technologies are not solely scientific but include cultural, educational, economic and political factors. A successful BNF-program, therefore, must involve, in addition to scientific research, efforts in training, education, outreach and technical assistance. Evaluations of socio-economical constraints are needed to publicize the benefits of BNF technology and provide advance warnings about potential difficulties, facilitating their removal.

Most farmers in Pakistan do not know that legumes fix nitrogen in their root nodules, yet traditional and modern farming of this country almost invariably includes legumes. The legume cultivation is a recognition by farmers over many centuries that legumes are valuable components in farming systems rather than intentional exploitation of biological nitrogen fixation per se.
The cost of inoculants is not usually a constraint to their use by farmers who are already spending money on seeds. Inoculant cost will seldom exceed 1% of the seed cost. However, for subsistence farmers who do not ordinarily purchase seed off the farm, the capital outlay for inoculant, albeit small, may be a disincentive to the use of inoculants.

BNF technology is a difficult technology to deliver by normal extension mechanisms. Thus a lack of illustrative and explanatory pamphlets and other aids, both for extension agents and farmers with whom they have contact, is also a constraint to implementation of BNF technology at the farm level. Furthermore, few of the senior administrators and decision makers who determine agricultural policy in Pakistan are fully aware of the opportunities for legume-based BNF technology in the agriculture sector of this country. Most policy makers are aware of some of the attributes of legumes, but relatively few appreciate the role played by biological nitrogen fixation in legumes. Among those few an even smaller proportion recognizes that it may be essential to employ specific technologies to ensure that nitrogen fixation occurs at all, let alone at a maximal rate. Thus there is a need for educational material, especially for this clientele group, bringing to their attention the real need to adapt currently available technology to the peculiar conditions of Pakistan.

The task of training and educating people to deliver BNF information and material has also been made difficult by the critical shortage of specialists with knowledge and interest in practical and applied aspects of the technology. This problem is compounded further as microbiologists and plant biologists from applied BNF leave the field for molecular biology and genetics, creating a serious void in programs addressing BNF needs of sustainable agriculture systems in developing countries like Pakistan.

Subsidies on nitrogen fertilizers in Pakistan has been a disincentive for farmers to use BNF products. However, as national debt rises and subsidies are phased out, BNF may become more attractive and acceptable. Moreover, crop subsidies and support programs are, in some countries like Pakistan, limited to cereal crops. This situation distorts the entire agricultural system. It provides a de-facto incentive to abandon legume-cereal crop rotations in favour of cereal mono-cropping.

Alleviating the Constraints: Some Suggestions for Future Research

As mentioned above, removing or alleviating the technical constraints on BNF applications in Pakistan would require a comprehensive research program. Identifying these limitations is a research task in itself, of which this paper has barely scratched the surface, and will naturally be the first step towards a better understanding and fuller implementation of this technology. To a certain extent, the process of identification and alleviation of constraints has been going on in the research labs of NARC, NIBGE and other institutes and universities involved in this field. Such activity needs to be continued, along with the determination of priorities and of new avenues of research. The main emphasis now should be on implementation of existing technology with modifications suited for the local environment. The following are some concrete recommendations towards alleviating some of the main constraints inhibiting the adoption of BNF technology, through better adaptation of the available methods.

Conduct Inoculation Trials

Legume inoculation should have a high priority in the research program of any institute active in this field. Legume inoculation field trials is one BNF technology that can be directly imported and utilized. However, since the majority of our farmers are illiterate and uninitiated, an educational effort is also needed to get farmers to practice this technology. Therefore, the need should first be demonstrated by definitive field trials. Such trials have been carried out on a limited scale, but have not been very extensive in nature. However, they should remain simple in design and small in size, so as to be easily comprehensible and replicable by farmers.

From the limited information on previous inoculation trials, it has been observed that while inoculation has been successful in a large number of instances, there have also been several cases of failure. The latter is indicated in a variety of ways such as the scarcity of nodules on the inoculated legume stands, inefficient nitrogen fixation capability, low nitrogen content of plant and overall low yield. Reasons for inoculation failures are many and varied, some of which have been mentioned previously in this paper, environmental and soil factors being the most important among them. Another reason for inoculation failures is the presence of a large population of indigenous rhizobia, less effective, but highly competitive and virulent in the soil. In this situation the plants are profusely nodulated but little nitrogen fixation takes place. In order to counteract these problems and to enhance the inoculation success, the quality of Rhizobium strains used in the inoculant production needs to be improved. This can be done through the selection of more effective and competitive strains. By selecting rhizobia with special characteristics such as tolerance to high temperature and acidity, these strains will compete better and survive in the soil. Thus research labs should continuously engage in expanding its rhizobial culture collection for this purpose.

Evaluate Soils and Crops for Potential of BNF Application

Research efforts should also be undertaken to evaluate the existing soil nitrogen content, the nitrogen fixing efficiency of indigenous rhizobium population (if any) and the overall soil environment (level of acidity, salinity etc.) where legumes are grown and areas where there is the potential for them to grow. Such an exercise will be helpful in evaluating whether inoculation is needed or not. Although the technology for such tests are available, they may end up costing more than the inoculant - and therefore may not be appropriate for small farmers. The soil tests do have a value for soil surveys of large areas where specific ecosystems or farm systems are evaluated. With sufficient samples, inferences about the need to inoculate can be made to other similar ecosystems and crop systems. The tests basically involve enumerating the population rhizobia in the soil at planting. If it is felt that the soil environment is too harsh for the inoculant to form nodules and fix nitrogen efficiently, better strains should be used or methods devised to genetically engineer rhizobium strains for an effective symbiosis to occur.

There should also be an effort to identify crops that can actually benefit from biofertilizer application. Although to a certain extent this has been done, there are still many areas where legumes have not been properly evaluated for their nitrogen fixing capability. The need here is for a comprehensive study, which will identify such crops in all major geographical areas to enable the full potential of biofertilizer application to be realized.

Design, Implement and Market a Better Rhizobium Inoculant Production System

There is only one legume inoculation production unit (at NARC) which at the present time is unoperational the unit is out of order. The unit was a "pilot plant" with the aim to be eventually transferred to the private sector, such a state does not attract many investors. Part of the reason why the unit is not working is a lack of maintenance of the facility, most probably arising from budgetary constraints. However, NARC still provides rhizobium inoculants (through the more conventional lab method). There is therefore a need to either revive the pilot plant at NARC or establish a new one. The production plant as developed by NifTAL is new and regarded as being more efficient and cost effective than the previous plants. Such a unit could be adapted for Pakistan, especially since it is not considered very costly.

Conduct a Cost-Benefit Analysis

A comprehensive benefit-cost analysis should be undertaken on the usage of BNF in general, and Rhizobium inoculants in particular. This should be a comparative study between nitrogenous fertilizers and biofertilizers, seeking to identify areas and crops where biofertilizer application will be beneficial economically, while at the same time sustaining crop yields. In evaluating the costs and benefits of the two types of fertilizers account will have to be taken of not only direct benefits and costs faced by the farmers but also the indirect benefits and costs, such as the protection of the environment and the costs to human health discussed earlier. Such a study will be important in framing appropriate government policies for the encouragement of the use of biofertilizers.

Emphasize Education and Training

Our farmers are not fully aware of the benefits gained from the application of biofertilizers. In fact most don't know the concept of biological nitrogen fixation, even though it may be occurring at some level in their crops. Therefore educating them about the process and technology is a step towards better utilization of biofertilizers. Education and training need not be confined to the farmers at the field, but should also be given to extension workers and motivators for the adoption of new farming methods. NGOs could also play a role by spreading this and other simple and inexpensive technologies to small and poor farmers and by popularizing science and technology among them.

Encourage Private Sector Involvement

The task of promotion of BNF can not be left completely to the government agencies and institutions currently active in the field, particularly when the government's budgetary position is weak and the role of the government is being restricted to more strategic objectives. It is, therefore, necessary to induce the private sector to become interested in implementing this technology at the farm level. The small-scale sector should be encouraged to build plants for the production of BNF inoculants, since the large-scale sector is likely to be more interested in capital-intensive chemical fertilizer plants. For this it may be given certain tax and other incentives by the government, at least in the initial period.

Presently, there are not many private sector manufacturing units that could possibly produce inoculant, and those that do not seem to be interested. The private sector may be willing to get involved once the market is established with prospects for growth. Moreover, investors may be skeptical of the actual contribution of biofertilizer to agricultural, probably due to their lack of awareness of BNF.

On the basis of data provided one can do the following estimation of the market for biofertilizers. As mentioned leguminous crops are grown on 2 million hectares of land (about 5 million acres); the Rhizobium inoculants that NARC produces cost (after government subsidy) 5 rupees per bag which are capable of fertilizing a fourth of an acre. Thus to fertilize one acre, 4 bags would be required costing 20 rupees. If only 10% of the 5 million acres are inoculated, then the potential market amounts to 10 million rupees. The actual cost of investment is not more than Rs 500,000

The government could provide incentive for private inoculant production by ways of soft loans. One approach could be to find people to start small businesses for this purpose. Most of the inoculant production in the U.S. is built in plants specifically for this purpose. In the beginning, they were small, garage or basement type operations, although there was a tendency to consolidate. A similar venture or ventures could be started in Pakistan, and be successful provided it receives the blessings of the government.

Another reason for encouraging the private sector is that government institutions will be more involved in research and not in production. The government could also then play the essential role of enforcing quality control standards. Quality control measures are necessary, otherwise the country risks having too many products on the market with no way for farmers to know which ones are good and which ones are worthless. Quality control procedures should be strict, but should not stifle initiative on the part of the procures. It is imperative that an independent agency keep a check on the products on the market. There are several institutions which are capable of enforcing such measures.

Conclusion

This paper has attempted to explore the possibilities of introducing an environmentally-friendly technology in a rather limited area of Pakistani agriculture. It is unlikely that BNF will make a major dent in the share of total fertilizer use in Pakistan, which is likely to continue to be dominated by chemical fertilizers in the foreseeable future, especially since the level of chemical fertilizer application remains relatively low compared to other countries. Nonetheless, there remains a need to promote environmentally sound technologies whenever available and to substitute those which are likely to prove hazardous to sustainable development. The BNF technology does offer such an opportunity in a vital area of agricultural production, viz. the leguminous crops, which form a substantial part of the diet of the poor and of the total agricultural production of the *barani* areas.

The paper has also attempted to investigate the limited success of BNF technology in Pakistan, inspite of the considerable research efforts by several institutions. The main difficulty seems to lie at the level of implementation and in the lack of an adequate incentive structure both for the production and use of BNF. The paper suggests a number of measures which could help reduce the constraints that stand in the way of a wider adoption of biofertilizer use in Pakistan.

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