

CROP INOCULANTS- AN UNDEREXPLORED POTENTIAL IN AGRICULTURE

Shahida N.Khokhar*

ABSTRACT:- Food security, poverty alleviation and environmental concerns have caused a shift in paradigm from use of agrochemicals to green way of life. Bioinoculants are in perfect harmony with the concept of Evergreen Revolution. They have the potential to improve value cost ratio (VCR) of agricultural production. Pakistan faces increase in degraded lands, water shortage, energy crisis, all contributing to poverty. During last two decades, many efforts were made to address the issue through developing and applying the inoculants. Several research institutions including National Agricultural Research Centre (NARC), Islamabad, Pakistan contributed significantly to these efforts. There are many reports on achieving increase in yield of legumes, cereals (wheat, maize) and vegetable crops. This however has yet to be taken to farmers' field effectively. The present paper reviews these efforts, lapses and unfolds new strategies in research and extension in future.

Key Words: Legumes; Cereals; Agriculture; Bioinoculants; Yield; Pakistan.

INTRODUCTION

Escalating population with steadily degrading land resource base poses serious threat to the agro-ecosystem and hence man himself in terms of food and energy. According to Millennium Development Goal II of FAO, number of hungry people (one thousand million) all over the world has to be halved by 2015. There is no option but developing a sustainable agriculture system. Improvement in agriculture sustainability requires optimized management of biological processes i.e., adopting management practices that enhance soil biological activity and thereby buildup long term soil productivity and health. Nature is already rendering worldwide ecosystem services, in the form of organic waste management, soil formation, bioremediation, nitrogen fixation etc. worth US \$ 1.54

billion. Nature had to be reinforced through judicious use of chemicals, enriching soil with active microbes. World is in need of Evergreen Revolution. This realization has caused a shift in paradigm from agrochemicals (fertilizers, pesticides) alone to integrated plant nutrient and pest management worldwide. Bioinoculants are a package of microorganisms, may be mounted on carrier and/or supplemented with additives (may be other natural or synthetic products) designed to provide benefits to crops. They are in perfect harmony with the concept of organic agriculture which promises evergreen revolution. They can play key role in this regard. Bioinoculants can be used in compound fertilizers either with organic material or chemical fertilizers or both or/and as biopesticides or bioremediators (Khalid and Khokhar, 2009).

*LRRI, National Agricultural Research Centre, Islamabad, Pakistan.
Corresponding author:sbb233@yahoo.com

World Overview

It has been 100 years since its appearance, rhizobial inoculant was the first to be classified as biofertilizer in the world, called America, with the market name of "Nitragin". Agricultural application of biofertilizer (microbial fertilizers) in China has been practiced since last 65 years.

In the sub-continent soil from legume field was used as fertilizing agent in other fields, centuries ago. Hundreds of products are now available in the countries around the world (Table 1).

Need of Inoculation in Pakistan

Crop legumes in Pakistan are though nodulated but mostly the nodules are ineffective and crops need inoculation with effective and competitive rhizobia. This is true in respect of chickpea, lentil, mungbean, mashbean and peas. Soybean is an introduced crop, it definitely requires inoculation.

Indigenous Soil Microbial Resources in Pakistan

Research in this direction started back in 1960s, at a very limited level. Nodulation status in legumes was studied in 1970s in southern Pakistan (Athar and Mahmood, 1978) and in Northern Pakistan (Khan, 1981). Artificial seed inoculation of 70 % chickpea crop area is needed. (Khattak et al. 2006). Occurrence of mycorrhizal roots in field grown cereals (Khan, 1974; Chaudhary et al., 2005) and actinomycetal nodulated non-legume herbs (e.g., *Datisca cannabina*), shrubs (e.g., *Coriaria nepalensis*) and trees (e.g., *Alnus*, *Hippophae*, *Eleagnus*) though had already been reported (Khokhar, 1978; Chaudhary et al., 1981), yet actinomycetes, being very slow growers in vitro could not get much popularity. Culturing of mycorrhizae and their inoculation has not yet been given due attention. Legumes remained focus till late 1990s. It was only 25 years ago, when beneficial

Table 1. Overview of global use of bioinoculants

Country	Product	Yield increase (%)	Crop
Australia	Nodulaid,EasyRhiz	10-30	Chickpea,clovers,
Brazil	Touchmercosur	Upto 60	Cereals
Canada	TagTeam Granular	10-50	Legume , cereals
China	NY227,	10-40	Legumes , cereals
India	VijayBio, and many others	4.7-51	Legumes and cereals
Japan	EM	10-60	Legumes, cereals
Pakistan	Biozote, Biopower, BM, Bakhtawar	10-40	Chickpea, mung, soybean, wheat, maize, rice
USA	Nitragin Gold, Dormal Plus, Soil Implant ,Biobrew	10-50	Soybean and many others

microbes other than mycorrhizae, e.g. rhizobia, Azorhizobia, Azospirilla, Azotobacter were found associated with cereals in nature. This encouraged several inoculation efforts (Nannipieri and Badalucco, 2003; Kennedy et al., 2004; Santa et al., 2004; Khokhar et al., 2002; Khokhar et al., 2006 a, b, 2007).

Pakistan needs Integrated Nutrient Management

Wheat, rice and maize are important food cereal crops, cultivated over 12.1 mha across Pakistan. Average yield of wheat, rice and maize being 2.4, 2.0 and 1.5 t ha⁻¹, respectively, depicts a yield gap of 50%, 70% and 80% for wheat, rice and maize, respectively. Legumes are grown on 1.40 mha in Pakistan. They too have yield as low as 0.5 t ha⁻¹. Much potential thus exists as to increase the yield. Energy crisis, and soaring prices of fertilizers during the past years made the farmers to run from post to pillar in search of fertilizers and those who got were threatened by lowered VCR (value cost ratio). Presently fertilizers are being used on only 30% of cultivated area. Shortfall of 0.1mt of urea has been indicated in 2011, which raised during the following years, thus incurring foreign exchange expenditure.

This situation demands a serious and well concerted effort to promote the use of bioinoculants for all crops, especially in subsistence farming. In fact this is a potential sector that can play role in poverty alleviation in poor farmers of marginal lands.

Several institutions in

Pakistan such as National Agricultural Research Centre (NARC) Islamabad; Nuclear Institute of Biology and Genetic Engineering (NIBGE), Faisalabad; Ayub Agricultural Research Institute (AARI), Faisalabad and University of Agriculture, Faisalabad (UAF) after performing basic and strategic research based on indigenous resources have launched farmers' field trials on legumes and cereals, which brought an increase of 10-40% in yield. However basis of inconsistency in inoculation response in cereals remains to be explored. It may originate in following ways.

Development of Formulation

Its includes medium of growth and age of culture

Microbial Physiology

Identification of factors responsible for switching on different modes of operation (nitrogen fixation, phosphorus solubilization, growth hormone production, exudation of antibiotic compounds) in the same microorganism is an area where meagre information is available.

Application Methodology

Seed biopriming or liquid inoculant (e.g. compost tea) or granular inoculants may perform better in dry ecologies where seed takes otherwise longer to germinate.

Soil Resident Microflora of Pakistan

It needs to be studied more intensely using molecular biology tools, to predict competitiveness of inoculant.

Cropping Sequence

Different cropping sequences lead to different plant root metabolites and ion exchange properties of soil, hence harbor different microflora. Survival, population buildup of one inoculant and its compatibility with following crop may be different in different cropping systems. Formulation should be developed and applied considering the specific growth habit of crop and site specific ecological conditions.

National Agricultural Research Centre (NARC) Islamabad started strategic research on development of indigenous bioferti-

lizer for legumes and establishment of pilot production plant in 1987, which is capable of producing inoculant for different legumes and cereals. After standardization and calibration of production procedure, in laboratory and executing field research trials, low cost Indigenous formulation so produced for chickpea was taken to farmers' field in 1994, when agreement with Engro Chemicals (Pvt.) Ltd was signed. Three years of trials on 15000 acres brought 10-40% yield increase at 60 locations on farmers' fields in Thal area (Table 2).

However it was not an attractive business for fertilizer

Table 2. Percent grain yield increase in various crops by NARC

Crop	Inoculant	Year	Sites	Increase over uninoculated unfertilized control (%)
Wheat	Azorhizobium	2009-2010 and 2010-2011	Islamabad, Sheikhpura, Quetta, D.I.Khan, Bahawalpur	25-50
Wheat GA2002	Azorhizobium	2007-08	Islamabad	40
Wheat GA2002	Azospirillum	2006-07	Islamabad	15-38
Wheat GA2002	Azospirillum + Rhizobium+ Pseudomonas	2006-07	Islamabad	27
Wheat MH97	Azorhizobium	1999-2000	Islamabad	40
Maize NARC3001	Azospirillum + Rhizobium+ Pseudomonas	2005	Tarlai, Islamabad	15-38
Maize kissan	Azospirillum	2001-2009	NARC, Islamabad	40
Chickpea	Rhizobium	1990,1994-96	Attock	20
			Thal area, Potowar dist, Sindh	10-40
Soybean	Bradyrhizobium	1994	Multan, Sahiwal, Quetta	40
Groundnut	Bradyrhizobium	1995	Attock, Chakwal, Rawalpindi	10-20

industry, reason being of their small margin in sale of the product. Realizing that a crop with greater human appeal should be undertaken, wheat, maize and rice were considered. After collection of material, isolates (Nitrogen fixers, phosphorus solubilizers, plant growth hormone producers, disease suppressant) obtained were evaluated under laboratory and glass house conditions. Field trials were carried out at NARC fields and outside for last ten years, under different soil/cultural conditions for different crops. Grain yield increase was 8-25%, in the presence of 50% of recommended urea-N and SSP-P wheat when compared with fertilized control and 15-100% when compared to unfertilized control (Table 2) at NARC, Islamabad (Khan et al., 2009; Khokhar et al., 2006b; Aslam et al., 2008). By now more than 250 institutions/agencies and hundreds of farmers have availed the inoculant.

NARC is playing its role with following mandate:

Maintaining cultures of indigenous diazotrophs (symbiotic, non-symbiotic), Phosphorus solubilizers (Hadmeed et al., 2004; Khokhar et al., 2006 b), plant growth promoting hormone producers (Aslam et al., 2008), collected from wide ecologies, i.e; D.I. Khan (Khokhar et al., 2006), Quetta, Potowar area (Khan et al., 1994), Thal (Khokhar and Khan, 1998; Khokhar et al., 2001) Kala Shah Kaku, Gujranwala, etc.

Value addition of bioinoculants

using compost.

Field evaluation trials at research centre and farmers' fields.

Dissemination of knowledge through training of scientists, agriculture extension workers, through media talks, farmer's field days and publication of brochures in Urdu and local languages

Collaboration with research and extension organizations.

This is not an uphill task? Collection of experiences pave way to success. Following gaps need to be filled.

Availability of Suitable Carrier

Carriers developed from industrial/agricultural waste materials, locally accessible, may be different in different regions of Pakistan can meet large scale demand, without imposing cost of transportation.

Quality Assurance

Different countries practice either of the models for quality control of biofertilizers Statutory Certification Norms (judicial) or Voluntary/Civil Certification Norms (consensus). China has an official organization, "Center for Quality Supervision and Test of Microbial Fertilizers for bioinoculant since 1995. International Federation of Organic Agriculture Movements (IFOAM), Bureau of Indian Standards (BIS), IOAM, CODEX, Soil Association of UK, Japan Agricultural Standards (JAS), National Organic Program (NOP) of USDA, National Association for Sustainable Agriculture (NASA) , Australia are some of the institutions operating in

USA, UK., Japan, Australia and India. Even while using unsterilized carriers, maximum threshold of contaminant should be less than 100g^{-1} .

Secrecy of Formulation

Number of publications on development of formulation is only 0.2% of the total literature on production, inoculation technologies and inoculation response. This also hinders the progress on improvement of formulation. Intellectual property rights however must be ensured at the same time.

Social Acceptability

There should be a better understanding between chemical and biofertilizer researchers to address the problem holistically. Even in western countries, where several biofertilizer companies exist, chemical fertilizer industry dominates. Farmer of rainfed area, where water availability is uncertain and poor man who cannot afford expensive chemical fertilizer can adapt this technology more easily. Vast field trials are needed.

Compatibility of Inoculant to Specific Field Ecology

It includes cropping sequence, soil resident microflora and climatic change. It is only through an understanding and integrating knowledge on plant interactions with inoculants in field that an effective and efficient inoculant can be developed. Microorganism inoculated to a crop may respond differently under different cropping sequence.

Farmer Friendly Product

Ease of application of a liquid inoculant either on the seed or in situ delivery has enhanced the popularity and use of liquid formulations in several countries in the last decade. Researchers have shown that the performance of liquid rhizobial formulations is comparable to that of peat-based products under field conditions (Hynes et al., 2001; Chilimba, 2008). Precoated seed can also be an option for short distance delivery. China and some European countries are using this technology in mountainous pastures.

FUTURE STRATEGIES

Immediate

Bring together indigenous knowledge, innovation and conventional science through participatory research methods.

Address the crops that have larger appeal to the farmers as well as to the population. Legumes are grown over 1.1 mha in Pakistan. There is a need to address cereal and other cash crops which occupy 17mha.

Design a concerted plan in formulation development and inoculation technology by multidisciplinary team, for delivery to end user in different agro-ecological zones of Pakistan. Agricultural microbiologists are core to a quality product.

Coordinate nation- wide for field trials and develop inoculation response prediction Models.

Joint public-private ventures are desirable.

Long Term

Soil N buildup and P availability need to be estimated in long term (5-10years) experiments.

Microbes having large ecological amplitude and saprophytic competence be selected.

Field resident microflora need to be ascertained qualitatively and quantitatively to address variability in response.

Endophytic associations be developed.

Precoated seeds/ liquid formulations/ foliar sprays should be considered.

Variety of waste products should also be considered as carrier, to make product cheap and survivable under ecology specific conditions.

LITERATURE CITED

- Aslam, M. Sultan T. and Saleemi, M. 2008. Use of nitrogen fixing PGPR for development of biofertilizers for crops of economic importance. Final Technical Report ALP-27. PARC, Islamabad.
- Athar, M. and Mahmood, A. 1978. A qualitative study of nodulating ability of legumes of Pakistan. *Pakistan. J. Bot.* 19: 95-99.
- Chaudhary, A.H. Khokhar S. N., Zafar, Y. and Hafeez, F. 1981. Actinomycetous root nodules in angiosperms of Pakistan. *Plant and Soil*, 64: 341-348.
- Chaudhary, M.S. Batool, Z. and Khan, A.G. 2005. Preliminary assessment of plant community structure and arbuscular mycorrhizas in rangeland habitats of Cholistan desert, Pakistan. *Mycorrhiza*, 15(8): 606-611.
- Chilimba, A.D.C. 2008. Evaluation of seed and liquid inoculation on Biological nitrogen fixation and grain yield of soybean. In: Dakora, F.D. Chimphango, S.B.M. Valentine, A.J. Elmerich, C. and Newton, W.E. (eds.) *Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture*. Proc. 15th Intern. Nitrogen Fixation Congress, 42:57-58.
- Hameed, S. Yasmin, S. Malik, K.A. Zafar, Y. and Hafeez, F. Y. 2004. *Rhizobium*, *Bradyrhizobium* and *Agro bacterium* strains isolated from cultivated legumes. *Biol. and Fertility Soil*, 39(3): 179-185.
- Hynes, R.K. Jans, D.C. Bremer, E. Lupwayi, N.Z. Rice, W.A. Clayton, G.W. and Collins, M.M. 2001. *Rhizobium* population dynamics in the pea rhizosphere of rhizobial inoculant strain applied in different formulations. *Can. J. Microbiol.* 47:595-600.
- Kennedy, I. R. Choudhury, A. T. M. A. and Mihály L. K. 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited? Faculty of Agriculture, Food and Natural Resources, SUNFix Centre for Nitrogen Fixation, The University of Sydney, Ross Street Building A03, Sydney, NSW 2006, Australia.
- Khalid, S. and Khokhar, S. N. 2009. Pesticides and bio-inoculants interacting with weeds In: Proc. International Thematic Workshop on 'Bioinoculant/ Bio-pesticide Production, Formulation and Application

- October 20-22, COMSTECH, Islamabad, Pakistan.
- Khan, A. G. 1974. Occurrence of Mycorrhizas in halophytes, hydrophytes and xerophytes and of endogone spores in adjacent soils. *J. Gen Microbiology*, 81:7-14.
- Khan, M.A. 1981. Some studies on the nodulation of wild and cultivated legumes from Northern Pakistan. M.Phil Dissertation, Quaid-e- Azam University, Islamabad.
- Khan, M.A. Khokhar S. N. Ashraf, M. Khan Y.H. and Aslam, M. 1994 Biological nitrogen and phosphorus resources in Pakistan-Promise and Prologue. *Pakistan J. Agric. Res.* 15(1):311-316.
- Khan, M.A, Khokhar, S.N. Ahmad, R. And Afzal, A. 2009 Wheat Growth and yield in response to coinoculation of Rhizobium, Azospirillum and Pseudomonas under rainfed conditions. *Intern. J. Biol. and Biotech.* 6(4):257-263
- Khattak, S.G. Khan, D.F. Shah, S.H. Madani, M.S. and Khan, T. 2006. Role of rhizobial inoculation in the production of chickpea crop. *Soil & Environ.* 25(2): 143-145.
- Khokhar, S.N. 1978 Survey of nonleguminous plants for root nodules. M. Phil thesis. Quaid-i-Azam University, Islamabad.
- Khokhar, S.N. and Khan, M.A. 1998 Spatial variability in rhizobial population in relation to soil moisture and other soil characters. *Emirates J. Agric. Sci.* 10:58-72.
- Khokhar, S.N. Khan M. A. and Chaudhry, M. F. 2001 Some characters of chickpea nodulating rhizobium native to Thal. *Pakistan J. Biol. Sci.* 4 (8): 1016-1018.
- Khokhar, S.N. Razzaq, A. and Majeed, A. 2002. Inoculation response of wheat to selected indigenous *Azorhizobium* spp. *Pakistan J. Agric. Res.* 17(4):320-325.
- Khokhar, S. N. Khan, M.A. and Afzal, A. 2006a. Natural endophytic diazotrophs associated with wheat roots in rainfed areas of Pakistan. Paper presented in Intern. Congr. Soil Sciences, Islamabad
- Khokhar, S.N. Khan, M.A. Afzal, A. and Ahmed, R. 2006b. Interaction of diazotrophs with phosphorus-solubilizing bacteria: Their effect on seed germination, growth and grain yield of maize, under rainfed conditions. *Int. J. Biol. Biotech.* 3(4): 773-777.
- Khokhar, S.N. Khan, M. A. Afzal, A. Ahmad R. and Sultan T. 2007. Improving root association of diazotrophs (*Azorhizobium*, *Azospirillum*) with wheat under rainfed conditions. Final Technical Report ALP project No. NR-18. PARC Islamabad.
- Nannipieri, P. and Badalucco, L. 2003. Biological processes. In: Bembi, D.K. and Neider, R. (eds). *Processes in Soil Plant System: Modelling Concepts and Applications*. The Haworth Press, Binghamton, NY.
- Santa, O. R. Dalla, R.F. Hernandez, G.L.M. Alvarez, P.R. Junior and Soccol, C.R. 2004. *Azospirillum* sp. inoculation in wheat, barley and oats seeds greenhouse experiments. *Brazilian Arch. Biol. and Technol.* 47(6): 843-850.