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Review Article

Biofertilizers in Pakistan: Initiatives and Limitations

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Abstract

Biological preparation containing live or latent cells of microorganisms or their metabolites, which when inoculated to seed, soil or roots of seedlings, promote plant growth and enhance harvestable yield is termed as biofertilizer. Biofertilizers, generally marketed, contain microbes capable of nitrogen (N) -fixation, phosphate solubilization/mineralization, phytohormone production and biocontrol. This paper reviews various initiatives in research and development of the biofertilizers taken by different institutes of Pakistan. The use of biofertilizers can play an important role in sustaining the agriculture systems. In Pakistan, where fertilizer is annually a hundred billion rupees business, even a 10% contribution by biofertilizer can save rupees ~10 billion. Various research groups/organizations are engaged in research and development on biofertilizers and have made their efforts to increase the application of biofertilizers in Pakistan's agriculture. Inconsistent field performance, lack of regulation and standards, quality issues of the product(s), awareness and lack of publicity are the major constraints in the widespread use of biofertilizers in Pakistan. If these problems are resolved, better results and responses can be expected through biofertilizer use in Pakistan. Assuring quality of products with extensive field-based testing, capacity building of resource persons and stakeholders on standard production processes, storage and application will help a wider adoption and popularization of the biofertilizer technologies.

Keywords: Biocontrol; Biofertilizer; N-fixation; Nutrients mobilization; Phosphate solubilization; Phytohormone production

Introduction

The word 'bio' stands 'life' and 'fertilizer' means 'a product providing nutrients in plant usable form'. Products (carrier or liquid based) containing living or dormant microbes (bacteria, actinomycetes, fungi, algae) alone or in combination, which help in fixing atmospheric N or solubilize/mobilize soil nutrients in addition to secretion of growth promoting substances for enhancing crop growth and yield are called biofertilizer. Unlike chemical fertilizers, biofertilizers are not used to provide nutrients present in them, except *Azolla*, which is used as green manure. Generally, biofertilizer are classified into four categories; (1) N_2-fixing biofertilizers e.g. sp. of *Rhizobium*, *Azotobacter*, *Azospirillum*, *Acetobacter*, blue green algae and *Azolla*, (2) P-solubilizing biofertilizer also called as phosphate solubilizing bacteria (PSB) or phosphate solubilizing microorganisms (PSM) e.g. some sp. of *Bacillus*, *Pseudomonas*, *Aspergillus* and vesicular arbuscular mycorrhiza (VAM) etc., (3) compost accelerator e.g. cellulolytic and lignolytic bacteria etc., and (4) plant growth promoting rhizobacteria (PGPR), promoting crop growth and yield through a variety of mechanisms e.g. *Azotobacter*, *Azospirillum*, *Rhizobium* in association with non-legumes, *Bacillus*, *Pseudomonas* etc.

The drastic use of chemical fertilizers in present agriculture has become a key source for high crop yields. The injudicious use of fertilizers is polluting the soil and environment at a faster rate. The sole reliance on chemical fertilizers [more than 156 million tons annually worldwide (Anonymous, 2012)] is not a sustainable and viable strategy because of the costs, both in domestic resource and foreign exchange involved in setting up fertilizer plants and sustaining the production. Thus, there is an acute need to have some cheaper way to exploit the intrinsic resources of the production system, which may be eco-and human-friendly. Biofertilizers may be important component of the integrated nutrient management; a cost effective and renewable resource management strategy.

A diverse array of bacteria including species of *Rhizobium*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Enterobacter*,

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Xanthomonas, *Serratia* and many others have been shown to promote plant growth (Vessey, 2003; Bashan *et al*., 2004). These PGPR can be used as biofertilizer and biocontrol agents (microbes capable to protect plants from infection by phyto-pathogens). It is not realistic to consider sustainable agriculture on a broad scale in the absence of biofertilizers. The production and application of biofertilizers for leguminous and non-leguminous crops are now common in developed world while in developing world it is now gaining pace. Pakistan is an agro-based country, largely dependent on agricultural products for its economy. Biofertilizers are used on farms at a limited scale which contributes only <2.0% of the total fertilizers used by the farmers for crop production (FAO, 2006).

However, there is a great potential to increase the use of biofertilizers for sustainable agriculture systems. Currently, Pakistan is spending huge amounts (i.e., Rs. > 100 billion) on the import and manufacturing of 8.41 million nutrient tons of chemical fertilizers (Anonymous, 2011). A 10% contribution by the biofertilizers to the total fertilizer consumption can save up to 10 billion rupees per year in Pakistan.

Various groups/organizations in Pakistan are working on biofertilizer research and development. They have reported significant increases in growth and yield of agronomically important crops in response to inoculation with microorganisms (Azad *et al*., 1991; Anwar, 1999; Aslam *et al*., 2000; Fatima, 2001; Asghar *et al*., 2002; Zahir *et al*., 2004, 2005, 2010a, b, 2011; Javaid, 2005; Khalid *et al*., 2006; Ahmad *et al*., 2008a; Shaharoona *et al*., 2006a, b, 2007, 2008; Mubeen *et al*., 2008; Naveed *et al*., 2008a, b; Qureshi *et al*., 2009a, b; Hussain *et al*., 2009; Ahmad *et al*., 2011, 2012, 2013a, b).

The extent of benefit from these biofertilizers depends upon their number and efficiency, which however, is governed by a large number of soil and environmental factors. The production technology for biofertilizers is relatively simple and installation cost is very low compared to chemical fertilizer plants. Besides, the long-term use of biofertilizers is economical, eco-friendly, more efficient, productive, and accessible to marginal and small farmers compared to chemical fertilizers. In the present review, we have discussed activities done so far on biofertilizers research and development by various research institution/organization in the country. We have also enlisted major limitations/recommendations in large scale production and popularization of the technology in the country.

History of Biofertilizers in Pakistan

Rhizobium is said to be known as the oldest biofertilizer in the world for leguminous crops and enricher of the soil fertility (Theophrastus, 372–287 BC, as reported by Danso, 1992). Classical concept of biological nitrogen fixation (BNF) was given by French chemist and agriculturist J.B. Boussingault in 1834, which was confirmed by Hellriegel and Wilfarth (1888). Isolation of *Rhizobium* (1888), *Azotobacter* (1901) and *Azospirillum* (1925) as N-fixing organisms were reported by Beijerinck. *Rhizobium* as N-fixer was first commercialized in 1895 under the name “Nitragin” in USA and was patented during 1896 by Noble and Hiltner (Bhattacharyya and Tandon, 2002). Microbial P-solubilization was first reported by Stalstrom (1903) and organisms were isolated by Pikovskaya in 1948 and Sperber in 1957.

In early 1920s, a quarter century before the creation of Pakistan, Punjab Agricultural College and Research Institute Lyallpur (the first Agricultural College in India), started bacteriological/BNF research by placing a researcher under the Agricultural Chemist. Later on, during 1926, BNF research and development efforts were strengthened with the creation of an independent post of “Agricultural Bacteriologist” at Punjab Agricultural College and Research Institute, Lyallpur. The bacteriological laboratory was established at Lyallpur in 1927 (Anonymous, 1930). The section designed both laboratory and field trials and executed simultaneously at Lyallpur and Gurdaspur to find out the efficacy of artificial inoculation for the economic cultivation of Egyptian clover (*Trifolium alexandrinum* L.). Inoculation studies were also conducted on chickpea (*Cicer aeretinum* L.), sweet clover (*Melilotus indica* L.), alfalfa (*Medicago sativa* L.), mungbean (*Vigna radiata* L.), mashbean (*V. mungo* L.), cluster bean (*Cyamopsis tetragonolobus* (L.) Taub.), and Persian clover (*T. resupinatum*) etc. Initial studies concluded that inoculum-treated seed produced a much heavier crop of better quality than that grown from the untreated seed (Anonymous, 1930). However, commercial *Rhizobium* inoculum production was started in 1956 in the region.

Use of Biofertilizers in Regional Agriculture

A major part of the developed (Australia, Canada, France, Italy, Netherland, New Zealand, South Africa, Sweden, United Kingdom and USA) as well as developing world (Argentina, Bangladesh, Brazil, Egypt, India, Indonesia, Kenya and Russia) has some level of commercial biofertilizer activities (FAO, 1991).

The activities of biofertilizers in Asian countries are being regularly looked after and promoted by Food and Agriculture Organization (FAO) and various other international agencies like Nitrogen fixation in Tropical Agricultural Legumes (NifTAL), Microbiological Resources Center (MERCIN), Forum for Nuclear Cooperation in Asia (FNCA) and Asian Productivity Organization (APO). India has a well established biofertilizer research and development infrastructure with over 169 operational biofertilizer production units. Industry has the capacity of 67,000 tons but was producing 39,000 tons of microbial formulations annually till 2007-2008 (NCOF Annual Reports, 2008). Product range includes

Rhizobium, *Azospirillum*, *Azotobacter*, *Azolla*, blue green algae, phosphate solubilizing microbes and other inoculants. Besides, some biocontrol products have also been released and produced in measurable quantity (Fuentes-Ramirez and Caballero-Mellado, 2005; Reddy and Saravanan, 2013; Reed and Glick, 2013).

China, the major rice growing country of the world is using rice biofertilizer *Azolla* on more than 6.5 million ha. The National Azolla Research Center at Fujian Academy of Agricultural Sciences is a leading institute in developing rice biofertilizer. Chinese are using plant growth promoting bacteria (PGPB) as biofertilizer for cereals and forages as well (Tien *et al*., 1979).

Japan, a long ago, started commercial production of N-fixing bacteria. Tokachi Federation of Agricultural Cooperatives (TFAC) is the major organization producing and distributing rhizobial biofertilizer since 1953. Products of arbuscular mycorrhizae as P-solubilizer and PGPR as biofertilizer and biocontrol are also available for the farming community. Similarly, FNCA, an international agency with its headquarters at Japan is actively working for biofertilizer promotion in Asian countries.

1. Biofertilizer Research and Development Vista in Pakistan

The uniqueness of microorganisms and their explored capabilities especially in a defined set of environmental and cultural conditions has made them potential candidates especially in agriculture along with other fields of life for resolving food security issues. Various research groups/organizations are working on biofertilizers research and development and have focused their efforts on increasing their role in Pakistan's agriculture. Research and development efforts are in the way to increase the role of biofertilizers in Pakistan as summarized in the following sections.

A. Ayyub Agricultural Research Institute (AARI), Faisalabad

Ayub Agricultural Research Institute, Faisalabad previously known as Punjab Agricultural Research Institute Lyallpur, a sister concern of Punjab Agricultural College Lyallpur is the oldest/pioneer center of BNF and biofertilizer research in the country. Research and development work was started in the early 1920s and was geared-up at different occasions. The scientists at AARI have been supplying bio-fertilizer under the trade name of "Associative Diazotrophs" (Egyptian clover, alfalfa, lentil (*Lens Culinaris* L.) etc.) since 1956.

The successful and consistent acceptance of *Rhizobium* cultures for legumes in the field (Azad *et al*., 1991) lead the Soil Bacteriology Section, AARI to work for beneficial microbial association prevailing in cereal and fodder crops and introduced *Azospirillum* and *Azotobacter* inoculants in early 1990s. In the mid 90s their consortia with the trade name "*Fasloon ka jarasimi teeka*" was released as a commercial product.

"*Fasloon ka jarasimi teeka*" containing Phosphate solubilizing microorganisms (PSM) was also introduced, which attracted a large number of farmers facing the problem of P-fertilizer shortages and high prices in the market. Data of more than 100 field trials showed the effect of rhizobial, associative diazotrophic and PSM inoculants on different leguminous and non-leguminous crops and in general, 20 ± 5% increase in yield of leguminous crops (*Medicago sativa* L., *Cicer arietinum* L., *V. radiata* L. and *Arachis hypogea* L.) with rhizobial inoculants, 13% increase in yield of cereals and fiber crops (wheat, maize, rice and cotton) with associative diazotrophic inoculants (*Azospirillum* and *Azotobacter* sp.) and 5% increase in yield of wheat and cotton with Phospho-bacterium inoculants (*Bacillus* and *Pseudomonas* sp.) was recorded (Annual Reports, AARI, Faisalabad, 2000-2011; Table 1-3).

Soil Bacteriology Section at AARI, Faisalabad is supplying inoculum to farmers of the province on a limited scale. During the current decades (2000-2011), section produced and supplied 38,800, carrier-based 250 g culture bags sufficient for inoculating 14,000 ha of crops (Annual Reports Soil Bacteriology Section, AARI, Faisalabad, 2000-2011).

B. Nuclear Institute of Agriculture and Biology (NIAB) and National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad

A very active BNF research group in Soil Biology Division was established at NIAB in 1972. The group comprised a galaxy of trained scientists, who published their work at national and international fronts. Some work on rice biofertilizer "*Azolla*" a water fern having N-fixing blue green algae (*Azolla-Anabaena*) as a symbiont in its leaves was also carried out in the country. The harsh summer climate during rice cultivation in the Punjab reduced its success and transfer to field whereas in northern rice growing areas (Swat Valley) of the country, technology found some pace because of mild and humid climate (Malik *et al*., 2000).

Later with the establishment of NIBGE, the team was placed at its "Biofertilizer Division" under the supervision of Dr. Kausar Abdullah Malik as Chief Scientific Officer in 1992. The institute was also awarded with local and foreign funding from International Atomic Energy Agency, International Centre for Genetic Engineering and Biotechnology and Islamic Development Bank to develop a Biofertilizer Resource Centre in the South Asian region. The efforts of the NIBGE scientists became fruitful in 1996 when they launched a biofertilizer with the commercial name of "BioPower". Species of the genus *Rhizobium* were used in legume biofertilizers i.e., for mashbean, chickpea, mungbean, soybean (*Glycine max* L.), alfalfa, pea (*Pisum sativum* L.) and cowpea (*V. unguiculata* L.);

Table 1: Rhizobial inoculation effect on yields of legumes

Crop	Location	Year	Average yield (kg ha^{-1})		Increase (%)
			Non-inoculated	Inoculated	
Barseem	FRI, Sargodha	2006-2007	68900	75700	9.9
Gram	BARI, Chakwal	2004-2005	839	890	6.1
		2005-2006	825	1005	21.9
		2006-2007	759	903	19.0
Mungbean	SSRI, Pindi Bhattian	2005	966	1246	29.0
		2006	743	897	20.7
		2007	738	895	21.3
	RARI, Bahawalpur	2008	741	846	14.0
Peanut	SAWCRI, Chakwal	2005	989	1232	24.5
		2006	1297	1641	26.2
		2007	862	943	09.4
	SAWCRI, Chakwal	2008	1119	1299	16.0

Source: Soil Bacteriology Section, AARI, reports (2004-2008),
FRI, Fodder Research Institute; BARI, Barani Agricultural Research Institute; SSRI, Soil Salinity Research Institute; RARAI, Regional Agricultural Research Institute; SAWCRI: Soil and Water Conservation Research Institute

Table 2: Effect of diazotrophs on yields of non-legumes

Crop	Year	Average Yield (kg ha^{-1})		Increase (%)
		Non-inoculated	Inoculated	
Wheat	2004-05 (04 trials)	4303	4331	3.00
	2005-06 (06 trials)	3546	3672	3.55
	2006-07 (07 trials)	4277	4645	8.61
	2008-09 (02 trials)	4189	4534	8.23
	2009-10 (01 trial)	5290	6200	17.20
	2010-11 (02 trial)	5080	6045	18.99
Rice	2005 (03 trials)	4129	4382	6.12
	2006 (02 trials)	3562	3783	6.19
	2007 (02 trials)	3392	3591	5.87
	2008 (04 trials)	3374	3644	8.00
	2009 (04 trials)	3413	3677	7.72
	2010 (02 trials)	3663	3992	6.79
	2011 (02 trials)	3584	4095	14.25
Seed Cotton	2005 (03 trials)	2412	2680	11.11
	2006 (04 trials)	2398	2594	08.17
	2007 (02 trials)	1616	1809	11.94
	2008 (02 trials)	1884	2091	10.99
	2009 (03 trials)	1928	2138	10.87
	2010 (01 trial)	2140	2439	13.97
	2011 (01 trial)	2185	2604	19.18

Source: Soil Bacteriology Section, AARI, reports (2004-2011)

Table 3: Effect of inoculation with phosphate-solubilizing bacteria on crop yields

Crop	Year	Average Yield (kg ha^{-1})		% increase
		Non-Inoculated	Inoculated	
Wheat	2004-2005 (03 trials)	2957	3092	4.56
	2005-2006 (02 trials)	3129	3245	4.00
	2006-2007 (01 trial)	3107	3249	4.57
	2009-2010 (01 trial)	4300	4800	11.62
	2010-11 (01 trial)	4250	4875	14.70
Seed Cotton	2005 (02 trials)	1621	1733	6.91
	2006 (02 trials)	1369	1446	5.62
	2007 (02 trials)	1160	1243	7.15
	2008 (02 trials)	2658	2787	4.85
	2009 (03 trials)	2325	2443	5.07
	2010 (01 trial)	1667	1750	5.0
	2011 (01 trial)	2125	2406	12.22
Maize (Fodder)	2005 (01 trial)	72500	82920	14.37
	2008 (02 trials)	51150	55850	09.19
	2010 (01 trial)	72000	74000	27.78
	2011 (01 trial)	6905	8094	17.22

Source: Soil Bacteriology Section, AARI, reports (2004-2011) whereas for cereals like wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryz sativa* L.), associative nitrogen fixing and PGPR were used. The group conducted some laboratory and field research on bacterial crop association and found that 40-70% of the nitrogen requirements of crop plants could be met through BNF (Hafeez *et al*., 1998) besides 60 to 80% improvement in crop yield.

After pot and field studies, biofertilizer "BioPower" was used on more than 11,000 hectares (7,000 of rice, 3,000 of legumes and 1,000 of wheat) of the Punjab during its first year of launch i.e. 1996-1997. The group claimed 50-70% saving in nitrogen fertilizer along with 20% increase in crop yield (NIBGE Activity Report, 1998; NFDC, 2008). While in the usage of "BioPower" at the time of wheat sowing, a saving of 30-50% chemical N fertilizer along with 15% increase in yield was also estimated (NIBGE Activity Report, 1998). Similarly, experiments were carried out to develop biofertilizer for maize. It was observed that "BioPower" with half recommended dose of NPK fertilizers gave the best results as compared to full recommended dose of NPK without "BioPower". For popularizing biofertilizers among the farmers, NIBGE involved business entrepreneurs of public and private sector (Ali Akbar Group, Pakistan Innovative Biotechnology Services and Pakistan Atomic Energy Commission) for transfer of production technology and proper training at the farm gate. Benefit-cost of the technology publicized that farmers can save substantial amount (upto 292 U$$ ha^{-1}) by using "BioPower" in various crops (NFDC, 2005).

NIBGE has a biofertilizer pilot production unit with notable strength of large and high quality fermentation, integrated production process, automatic mixing, filling and packaging system and experienced personnel to monitor the production. The pilot project has been designed to upscale the biofertilizer production in near future to meet the expanding demand (Hameed, 2009).

NIBGE has supplied "BioPower" between 9000 to 12000 ha of crops annually during the last ten years, but the inconsistency of field results led the team to divert their research focus and reduce the "Biofertilizer Division" to a small section of "Plant Microbiology Division" with other areas identified to work.

C. National Agricultural Research Centre (NARC), Islamabad

Soil Biology and Biochemistry Division in Land Resources Research Program of NARC started research on N_2 fixation in legumes during early 1980's. They tested some imported rhizobial strains (from NifTAL, Hawaii) for their effects on the yield of legumes in Pakistan. Later on, local *Rhizobium* spp. was isolated for inoculating legume crops of agronomic importance (chickpea, lentil, mungbean, mashbean, soybean, groundnut (*Arachis hypogaea* L.), pea, Egyptian clover, sesbania and alfalfa). More than 200 isolates of different rhizobia are available in the "*Rhizobium* Gene Bank" of NARC. The center launched a biofertilizer product in 1990 under the name of "Biozote". The efficacy of the "Biozote" was tested on a large scale for various legume crops during late 1990's and about 60,000 packets of the product were supplied to farmers during a three year project in collaboration with Pakistan Agricultural Research Council (PARC) and Engro-Chemical Pakistan Ltd (ECPL). On the basis of data collected from 300 farmer fields and 80 field demonstrations, 20-50% increase in yield for all legume crops were obtained with the application of "Biozote" only (Silver Jubilee Report NARC, 2006). Furthermore, it was claimed that benefit-cost ratio of the technology was 30:1 and if applied on 50% legume area it could add Rs. 4.0 billion to the national economy through increased production (Silver Jubilee Report NARC, 2006).

The center has a pilot production unit "Legume Inoculum Production Unit (LIPU)", capable of producing more than 150,000 culture bags annually. Currently, the center is providing approximately 3,000 culture bags to the farming community (personal communication) for inoculating legume and cereal crops per annum.

D. Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad

The Department of Soil Science at University of Agriculture, Faisalabad was upgraded to the status of "Institute of Soil and Environmental Sciences" in 2003. The institute is actively engaged in basic and applied research on soil microbiology and biochemistry along with some other specialties.

The soil microbiologists working at the institute have successfully isolated various beneficial bacteria and have developed certain microbial formulations for use as biofertilizers. They have also proposed to utilize microbial metabolites pertaining to biologically active substances or plant growth regulators which seem to be one of the best options for plant growth improvement rather than using living cells of inoculants under Pakistan agricultural conditions (Zahir *et al*., 2004; Khalid *et al*., 2006). Group is pioneer in the region, using microbial metabolites and getting good results in terms of growth and yield promotion of various crops. They developed a microbial metabolite based biofertilizer namely "Rice-Biofert", a liquid preparation in 2002. Three-year cumulative data of twenty multi-location trials inferred an increased rice yield up to 20%.

Various cultures of *Azotobacter* have also been isolated from different soils and their effectiveness for increasing crop yields has been extensively studied by the Soil Microbiology and Biochemistry Group. Similarly, other PGPR i.e. *Pseudomonas*, *Burkholderia*, *Bacillus*, *Serratia* etc. have also been isolated, which appreciably proved their worth as plant growth promoter.

A number of PGPR strains possess the enzyme ACC-deaminase that hydrolyses ACC (ethylene precursor) into ammonia and α-ketobutyrate. Thus, PGPR containing ACC-deaminase, when colonized on plant roots, can act as a sink for ACC and thereby lower the plant ethylene. This eliminates or reduces the potential inhibitory effect of high ethylene concentrations and may allow plants to develop a better root system. Plants treated with PGPR containing ACC-deaminase are more resistant to the deleterious effects of stress ethylene synthesized by various environmental stresses like salinity, drought, heavy metals etc. (Mayak *et al*., 2004a, b; Nadeem *et al*., 2007, 2009, 2010; Zahir *et al*., 2008, 2009).

Biofertilizers based on ACC-deaminase biotechnology developed by ISES have shown their worth even under stress conditions including drought and salinity (Nadeem *et al*., 2006a, b, 2007, 2009, 2010, 2013; Arshad *et al*., 2008; Zahir *et al*., 2008, 2009; Shaharoona *et al*., 2006a, b, 2007, 2008; Naveed *et al*., 2008a, b; Ahmad *et al*., 2011, 2012, 2013a, b). In order to promote biofertilizers at farmer's field, ISES has conducted number of demonstration trials at farmer's field to display the potential of biofertilizer "UniGrow", a PGPR-enriched organic formulation and got highly encouraging results (Ahmad *et al*., 2008b; Naveed *et al*., 2008b; Shahzad *et al*., 2008). Studies suggested that an integrated use of bio, chemical and organic fertilizers could be very effective for getting higher yields to meet increasing-demand of food in the country. Encouraging results of the rhizobial inoculation on non-legumes and synergistic effect of precursor-inoculum interaction on legumes have been assessed under field conditions (Mehboob *et al*., 2008, 2011, 2012; Hussain *et al*., 2009; Zahir *et al*., 2000, 2005, 2010). Recently in 2011, "Rhizogold" (RG), a mix-culture of *Rhizobium* and ACC-deaminase containing PGPR has been formulated by ISES enhancing legume yields by 40-45%. Very recent, "Rhizogold^{Plus}" (RG^{+}), a multi-strain biofertilizer developed from the efficient strains of PGPR containing ACC-deaminase to mitigate the impact of salinity stress on cereals (Jamshaid *et al*., 2014; Khan *et al*., 2014). Currently, both products are under extensive evaluation at farmer's field in the ALP/HEC funded projects.

E. Nature Farming Research and Development Foundation (NFRDF)

Effective microorganism (EM) technology was imported by a former Professor of Soil Science, University of Agriculture, Faisalabad from Dr. Teruo Higa of Japan as biological input for sustainable yields. Nature Farming Research Centre was established at UAF to work on the technology.

He further developed beneficial microorganism (BM) Technology, with a claim to enhance the soil productivity by the use of beneficial microbes along with crop residues, livestock manures, industrial wastes, compost from urban refuses, green manures and other nitrogen-binding crops thus reducing the need for costly synthetic chemical fertilizers. This technology is being tested to increase the yield of agronomically important crops. A new innovation of BM fermenter/super fermenter models have been established for taking greater benefit of smaller quantities of available organic matter and using brackish water for irrigation through BM-Technology (Hussain, 2008).

NFRDF had run various projects at farmer's field (Kurrianwala, Faisalabad, 1994-2003; Asim Agri. Farm, Tando Allayar, Sindh, 1997-2007; Shezan (M-Benz) Farms, Nawazabad, Mirpurkhas, Sindh, 1995-2007) and sugar mills of the Punjab (Shakarganj Sugar Mills Ltd., Jhang, Brother Sugar Mills Chunian, Kasur, 2004-2007) to evaluate the efficiency of this technology for maintaining long-term soil fertility, promote sustainable use of soil and other inputs and also minimize pollution, increase soil biological activity and on site recycling of plant and animal origin waste. NFRDF field experimentation concluded as (1) More biological activity, (2) Sugarcane yield varied from 73.5 up to 196 tons ha^{-1}, (3) Average increase in profit/ha due to BM technology varied from rupees 39,621/- to 66,672/- and (4) Successful reclamation and development of inferior quality soil and water resources (Hussain, 2008).

A number of farmers are now using EM-Technology for crop production in the Punjab. EM-BIOAAB is used for crop production, fish farming and environments; EM-BIOVET is used for animal and poultry production while EM-BIOCONTROL, which is not a pesticide, is used for controlling insect/pest diseases of crops, vegetables and fruits (Hussain and Higa, 2001; Hussain, 2008).

F. Research in Various Institutes of Higher Learning

Research on soil microbial potentials and plant microbe interactions to see their effect on soil and plant health is under way at various centers of higher learning in the country. Karachi University, Karachi; Quaid-i-Azam University, Islamabad; Comsat University, Islamabad; PMAS-Arid Agriculture University, Rawalpindi; Punjab University, Lahore; Bahauddin Zakariya University, Multan; the Islamia University, Bahawalpur; KPK Agriculture University, Peshawar; AJK University, Rawalakot have made some marvelous achievements. A large quantity of work has been published in national as well as international journals. Areas explored include *Rhizobium* (symbiotic and non-symbiotic) associations with legumes and non-legumes (Hayat, 2005; Zafar-ul-Hye, 2008; Mehboob *et al*., 2008, 2011, 2012; Hussain *et al*., 2009; Zahir *et al*., 2010a, b, 2011), isolation and characterization of various microbial sp. for disease control (Ghaffar, 1988; Ehtesham-ul-Haque, 1994; Siddiqui, 2000; Siddiqui and Shaukat, 2002; Dawar *et al*., 2005; Sheikh *et al*., 2006; Mehboob *et al*., 2008) and plant growth promotion (Anwar, 1999; Fatima, 2001; Khalid, 2001; Khalid *et al*., 2004; Mehboob *et al*., 2011, 2012; Naveed *et al*., 2008a, b; Shakir, 2008; Qureshi *et al*., 2009b; Hussain *et* *al*., 2009). Microflora providing relief against different stresses (Bilal, 1998; Nadeem *et al*., 2007, 2009, 2010; Saleem *et al*., 2007; Arshad *et al*., 2008; Zahir *et al*., 2008, 2009, 2010a, Ahmad *et al*., 2011, 2012, 2013a, b), microbial production of phytohormones (Zahir *et al*., 2004; Khalid *et al*., 2006; Shakir, 2008; Afzal and Bano, 2008; Qureshi *et al*., 2013), exploitation of bacterial and fungal communities for better soil and plant health, estimation of genetic variation and developing molecular markers for measuring and improving microbial efficiencies (Hameed, 2003), phytoremediation of soil and environmental toxicants (Iqbal, 1994; Talat, 2000; Faryal, 2003; Faisal, 2004; Siddique *et al*., 2003; Hussain *et al*., 2007; Ahmad *et al*., 2013; Asghar *et al*., 2013), isolation and characterization of PGPR and their potential use as biofertilizer have also become priority research areas (Javaid, 2005). Research work done in various universities is of academic nature, which provides guidelines for applied research. In this way, a good interaction between research and educational institutes leads to translate scientific ideas to realities.

2. Limitations in the Mass Scale Production and Commercialization of Biofertilizers in Pakistan

Though microbial technologies have proven their worth when applied to various agricultural and environmental problems with considerable success over the past 50 years but have not been widely accepted at large scale because it is often difficult to consistently reproduce their beneficial effects in a varied set of conditions prevailing in above and below ground environment.

Major limitations in large scale production and popularization of the technology in the country are listed below:

1. Lack of regulation and standards for biofertilizer. In Pakistan, regulations for production and commercialization of biofertilizers have not yet been framed at national level. Hence, sub-standard inoculants is one of the most important limitations causing their failure in field and adversely affecting the farmer's confidence in the product.
2. Inadequate awareness among the farming community about bio-inoculants.
3. In most cases, biofertilizers are not ecological, environmental and crop/cultivar specific because of which biofertilizers remain unable to show expected positive results. Ultimately, the convinced farmer looses its confidence in the technology.
4. Lack of strong promotion and extension work and publicity among the end users.
5. Lack of trained man power and cost involved in the production of quality biofertilizer.
6. Lack of storage and transportation facilities in the country to avoid contamination.
7. Extreme climatic conditions (especially prolonged hot and dry summer) often make the results of biofertilizers inconsistent.
8. Low organic matter content of soil in semi arid regions like Pakistan does not allow the beneficial microorganisms to persist and interact positively with plant species.
9. Lack/poor availability of suitable carrier materials for biofertilizer formulation.
10. Poor labeling (not giving genus name, viable cell number and expiry date etc.) of biofertilizer products also shatter their credibility.

3. Future Prospects and Recommendations

For a more comprehensive development and utilization of biofertilizers, there are several issues/recommendations, which need to be taken care by the Government in the future research.

1. Necessary legislation for monitoring biofertilizers, their quality and hazardous effects, if any, on plants and human beings. This is a very critical issue that should be assessed and demands collaborative efforts between the public and private sectors.
2. Government may subsidize or provide loans for small scale production units of biofertilizers at local level.
3. There is dire need of culture collections (CC)/gene bank of microorganisms in the country. All the characterized microbes/potential biofertilizers candidates from different institute and individual scientist may be pooled, preserved (freeze drying lypholization), given accession numbers, identified globally acceptable molecular tagging like 16S or housekeeping genes and validation chemotaxnomically if further required.
4. Research focus more on soil microbiomes, soil metagenomics, transcriptomics and key genes of active microorganisms associated with nodulation, growth regulators, disease suppressing and nutrient cycling in soil.
5. A strong extension and training program may be initiated for motivating the farmers and other concerned personnel to exploit full potential of the biofertilizer technology. The task may be given to the proposed Microbiology Research Labs in each Agro-ecological zone or a crash program may be launched through extension departments. Short term diploma programs may also be initiated at agriculture universities or proposed Microbiology Research Centers/Labs for developing biofertilizer for their local conditions using the microbial strains from Microbial Resource Centers.
6. Development of biofertilizers using microbial consortia containing effective, competitive and stress tolerant microbial strains.
7. The potential of biofertilizers to supply micronutrients and for biofortification of food crop also is to be explored.
8. Lesser used biofertilizers, such as phosphate solubilizing microorganisms (PSMs) and phosphate mobilizers like VAM are promising in supplying phosphorus and other micronutrients. In this direction culture of obligate symbiont (VAM) under laboratory conditions will enable testing of their performance in field

conditions. Genetic basis for competitive superiority further needs to be worked out.

9. Selection of an economical synthetic carrier capable of maintaining high viable count and improvement in inoculation techniques to ensure establishment and persistence in soil.

10. Development of polymicrobial biofertilizers e.g., PGPR, Rhizobia, PSMs, VAM.

11. Bioconversion of organic wastes into value added organic/biofertilizers at local level.

12. Research is also needed to improve the endophyte - host plant interaction through endophyte molecular breeding. Compared with crop genetic engineering, the engineering of endophytic bacteria should be a much easier process. The genetic modification of endophytes with useful genes will impart new traits into host plants inoculated with these bacteria.

13. Impregnated fertilizers i.e. the chemical fertilizers coated with promising microbial strains could be the beginning of a new understanding about the natural/synthetic sources of nutrition potentially leading to a better understanding of the “microbial-enhanced fertilizer use efficiency”.

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Reference

Afzal, A. and A. Bano, 2008. *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *Int. J. Agric. Biol.,* 10: 85–88

Ahmad, I., M.J. Akhtar, H.N. Asghar and Z.A. Zahir, 2013. Comparative efficacy of growth media in causing cadmium toxicity to wheat at seed germination stage. *Int. J. Agric. Biol.,* 15: 517–522

Ahmad, M., Z.A. Zahir, H.N. Asghar and M. Arshad, 2012. The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. *Ann. Microbiol.,* 62: 1321–1330

Ahmad, M., Z.A. Zahir, H.N. Asghar and M. Asghar, 2011. Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant-growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate-deaminase. *Can. J. Microbiol.,* 57: 578–589

Ahmad, M., Z.A. Zahir, M. Khalid, F. Nazli and M. Arshad, 2013a. Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer’s fields. *Plant Physiol. Biochem.,* 63: 170–176

Ahmad, M., Z.A. Zahir, S.M. Nadeem, F. Nazli, M. Jamil and M. Khalid, 2013b. Field evaluation of *Rhizobium* and Pseudomonas strains to improve growth, nodulation and yield of mung bean under salt-affected conditions. *Soil Environ.*, 32: 158–166

Ahmad, R., M. Arshad, A. Khalid and Z.A. Zahir, 2008a. Effectiveness of organic/biofertilizer supplemented with chemical fertilizers for improving soil water retention, aggregate stability, growth and nutrients uptake of maize (*Zea mays* L.). *J. Sustain. Agric.,* 31: 57–77

Ahmad, R., M. Arshad, Z.A. Zahir, M. Naveed, M. Khalid and H.N. Asghar, 2008b. Integrating N-enriched compost with biologically active substances for improving growth and yield of cereals. *Pak. J. Bot.,* 40: 283–293

Annual Reports, 2000-2011. Soil Bacteriology Section, Ayub Agriculture Research Institute, AARI, Faisalabad, Pakistan

Anonymous, 1930. Guide to the Punjab Agricultural College and Research Institute, Lyallpur. Printed by the Superintendent, Government Printing, Punjab, Lahore, Pakistan

Anonymous, 2012. Fertilizer Consumption Statistics. Available on: http://www.fertilizer.org/ifa/statistics.asp (accessed on: February 17, 2012)

Anonymous, 2011. *Economic Survey 2010-2011*. Government of Pakistan, Finance Division. Economic Adviser Wing, Islamabad, Pakistan

Anwar, G., 1999. Production of growth hormones and nitrogenase by diazotrophic bacteria and their effect on plant growth. *Ph.D. Thesis*. University of the Punjab, Lahore, Pakistan

Arshad, M., B. Shaharoona and T. Mahmood, 2008. Inoculation with *Pseudomonas* spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). *Pedosphere,* 18: 611–620

Asghar H.N., M. A. Zafar, M. Y.Khan and Z.A. Zahir, 2013. Inoculation with ACC-deaminase containing bacteria to improve plant growth in petroleum contaminated soil. *Romanian Agric. Res.,* 30:1–9. DII 2067-5720 RAR 2012-254

Asghar, H.N., Z.A. Zahir, M. Arshad and A. Khaliq, 2002. Relationship between *In vitro* production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biol. Fertil. Soils,* 35: 231–237

Aslam, M., I.A. Mehmood, T. Sultan and S. Ahmad, 2000. Inoculation approach to legume crops and their production assessment in Pakistan. A review. *Pak. J. Biol. Sci.,* 3: 193–195

Azad, M.I., M.S. Dogar and M.A. Shakir, 1991. Improvement in yield of legumes with rhizobial inoculation. *In*: *Proceedings “Awareness Seminars (Part-1),* pp: 226–232. Directorate of Agricultural Information, Punjab, Lahore, Pakistan

Bashan, Y., G. Holguin and L.E. de-Bashan, 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances (1997-2003). *Can. J. Microbiol.,* 50: 521–577

Beijerinck, M.W., 1888. Die bacterien der papilionaceen knollchen. *Botanische Zeitung,* 46: 726–804

Beijerinck, M.W., 1901. Uber oligonitrophile mikroben. Zbl. *Bacteriol, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II.,* 7: 561–582

Beijerinck, M.W., 1925. Ube rein Spirillum, Welches frein Stickstoff binden Kann? Zbl. *Bacteriol, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II.,* 63: 353–359

Bhattacharyya, P. and H.L.S. Tendon, 2002. *Dictionary of Biofertilizers and Organic Fertilizers*. Fertilizer Development and Consultation Organization, New Delhi, India

Bilal, R., 1998. Associative biological nitrogen fixation in plants growing in saline soils. *Ph.D. Thesis*. Department of Botany, University of the Punjab, Lahore

Danso, S.K.A., 1992. Biological nitrogen fixation in tropical agrosystem, twenty years of biological nitrogen fixation research in Africa. *In*: *Biological Nitrogen Fixation and Sustainability of Tropical Agriculture*, p: 3. Mulongoy, K., M. Gueye and D.S.C. Spencer (eds.)

Dawar, S., F. Perveen and A. Dawar, 2005. Effect of different strains of *Rhizobium* spp. in the control of root infecting fungi and growth of crop plants. *Int. J. Biol. Biotechnol.,* 2: 415–418

Ehtesham-ul-Haque, S., 1994. Use of rhizobia in the control of soil born plant disease caused by root infecting fungi. *Ph.D. Thesis*. Department of Botany, University of the Karachi, Karachi, Pakistan

Faisal, M., 2004. Screening of chromium resistant bacteria for detoxification potential and plant growth stimulation under chromium stresses. *Ph.D. Thesis*. Department of Botany, University of the Punjab, Lahore, Pakistan

FAO, 2006. *Plant Nutrition for Food Security: A Guide for Integrated Nutrient Management*, FAO Fertilizer and Plant Nutrition Bulletin 16, Food And Agriculture Organization Of The United Nations Rome, Italy

FAO, 1991. *Organic Recycling in Asia and the Pacific*. Regional office for Asia and Pacific (RAPA)/FAO, Bangkok, Thailand

Faryal, R., 2003. Role of microorganisms in bioremediation of heavy metal ions and organic pollutants present in textile industry. *Ph.D. Thesis*. Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Fatima, Z., 2001. Response of chickpea to plant growth regulators; contribution of nitrogen -fixed by chickpea to following wheat crop. *Ph.D. Thesis.* Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Fuentes-Ramirez, L.E. and J. Caballero-Mellado, 2005. Bacterial biofertilizers. *In*: *PGPR: Biocontrol and biofertilization,* pp: 143–172. Siddiqui, Z.A. (ed.). Springer, Dordrecht, The Netherlands

Ghaffar, A., 1988. Biological control of sclerotial diseases. *In*: *Biocontrol of Plant Diseases*, pp: 153–175. Mukerji, K.G. and K.L. Garg (eds.). CRC Press Inc. Boca Raton, Florida, USA

Hafeez, F.Y., T. Ahmad, S. Hameed, S.K.A. Danso and K.A. Malik, 1998. Comparison of direct and indirect methods of measuring nitrogen fixation in field grown chickpea genotypes. *Pak. J. Bot.,* 30: 199–207

Hameed, S., 2003. Molecular characterization and significance of rhizobial exopolysaccharides. *Ph.D. Thesis*. Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Hameed, S., 2009. *BioPower: Biofertilizer Technology-A commercial venture of NIBGE.* Pp. 38-40. In: National Conference on "Development and Commercialization of Biotechnology Products" June 19-20, 2009. Jointly organized by National Commission on Biotechnology and National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

Hayat, R., 2005. Sustainable legume-cereal cropping system through management of biological nitrogen fixation in pothwar. *Ph.D. Thesis*. Department of Soil Science, Soil Water Conservation, University Arid Agriculture, Rawalpindi, Pakistan

Hellriegel, H. and H. Wilfarth, 1888. *Untersuchungen ueber die stickstoffnahrung der Gramineen and Leguminossen*, p: 234. Beilageheft Zn der Zeitschrift des Verein Rubenzucker – Industrie Deutschen Reichs, Germany

Hussain, M.B., I. Mehboob, Z.A. Zahir, M. Naveed and H.N. Asghar, 2009. Potential of *Rhizobium* spp. for improving growth and yield of rice (*Oryza sativa* L.). *Soil Environ.,* 28: 49–55

Hussain, S., M. Arshad, M. Saleem and A. Khalid, 2007. Biodegradation of α- and β-endosulfan by soil bacteria. *Biodegradation,* 18: 731–740

Hussain, T., 2008. Soil care for sustainable environment through fermenter and biogenerator systems using efficient microorganisms technology. *In*: *Proceeding of the National Seminar "Soil Care for Sustainable Environment"*, pp: 155–163. Zahir, Z.A., M. Arshad and A.S. Bhatti (eds.). May 16-17, 2006. Inst. Soil Environ. Sci., Univ. Agri., Faisalabad, Pakistan

Hussain, T. and T. Higa, 2001. *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment,* p: 216. Nature Farming Research Center, Univ. Agri., Faisalabad, Pakistan

Iqbal, M.J., 1994. Isolation and characterization oil degrading microorganisms and their possible role in enhanced oil recovery. *Ph.D. Thesis*. Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Jamshaid, M.U., Z.A. Zahir, H.N. Asghar, M.Y., Khan, M. Naveed, M.J. Akhtar and M. Arshad, 2014. Development of a multi-strain biofertilizer "Rhizogold" for sustainable production of pulses on marginal lands of Pakistan. p: 247. *In*: 15th International Congress of Soil Science, 18-20 March, 2014, NARC, Islamabad, Pakistan

Javaid, A., 2005. Prospects of EM and VAM technology for improving growth, yield and nitrogen fixation in *Vigna radiata* (L.) Wilczek. *Ph.D. Thesis*. Department of Botany, University of the Punjab, Lahore, Pakistan

Khalid, A., 2001. Potential of auxin producing plant growth promoting rhizobacteria (PGPR) for improving growth and yield of cereals. *Ph.D. Thesis.* Institute of Soil Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Khalid, A., M. Arshad and Z.A. Zahir, 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.,* 96: 473–480

Khalid, A., M. Arshad and Z.A. Zahir, 2006. Phytohormones: microbial production and applications. *In*: *Biological Approaches to Sustainable Soil Systems,* pp: 207–220. Uphoff, N., A.S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, J. Pretty, P. Sanchez, N. Sanginga and J. Thies (eds.). Taylor & Francis/CRC, Boca Raton, Florida, USA

Khan, M.Y., Z.A. Zahir, H.N. Asghar, M.U. Jamshaid, M. Naveed, M.J. Akhtar and M. Arshad, 2014. RhizogoldPlus: A multi-strain biofertilizer for sustainable production of cereals from salt affected soils. p: 245. *In*: 15th International Congress of Soil Science, 18-20 March, 2014, NARC, Islamabad, Pakistan

Malik, K.A., S. Miza, S. Melnaz and G. Rasul, 2000. The role of plant-associated beneficial bacteria in rice-wheat cropping system. *Abstracts 8th International Symposium on Nitrogen Fixation with Non-legumes,* p: 46

Mayak, S., T. Tirosh and B.R. Glick, 2004a. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.,* 42: 565–572

Mayak, S., T. Tirosh and B.R. Glick, 2004b. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sci.,* 166: 525–530

Mehboob, I., Z.A. Zahir, A. Mahboob, S.M. Shahzad, A. Jawad and M. Arshad, 2008. Preliminary screening of *rhizobium* isolates for improving growth of maize seedlings under axenic conditions. *Soil Environ.,* 27: 64–71

Mehboob, I., Z.A. Zahir, M. Arshad, A. Tanveer and F. Azam, 2011. Growth promoting activities of different *Rhizobium* spp., in wheat. *Pak. J. Bot.,* 43: 1643–1650

Mehboob, I., Z.A. Zahir, M. Arshad, A. Tanveer and M. Khalid, 2012. Comparative effectiveness of different *Rhizobium* sp. for improving growth and yield of maize (*Zea mays* L.). *Soil Environ.,* 31: 37–46

Mubeen, F., A. Aslam, V. Radi, M. Schloter, K.A. Malik and F.Y. Hafeez, 2008. Role of Nature's fertility partners with crop protectants for sustainable agriculture. *In*: *Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture.* Dakora, F.D., S.B.M. Chimphango, A.J. Valentine, C. Elmerich and W.E. Newton (eds.). Springer, The Netherlands

Nadeem, S.M., I. Hussain, M. Naveed, H.N. Ashgar, Z.A. Zahir and M. Arshad, 2006a. Performance of plant growth promoting rhizobacteria containing ACC-deaminase activity for improving growth of maize under salt-stressed conditions. *Pak. J. Agric. Sci.,* 43: 114–121

Nadeem, S.M., Z.A. Zahir, M. Naveed and M. Arshad, 2007. Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. *Can. J. Microbiol.,* 53: 1141–1149

Nadeem, S.M., Z.A. Zahir, M. Naveed and M. Arshad, 2009. Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. *Can. J. Microbiol.,* 55: 1302–1309

Nadeem, S.M., Z.A. Zahir, M. Naveed and S. Nawaz, 2013. Mitigation of salinity induced negative impact on growth and yield of wheat by plant growth promoting rhizobacteria in naturally saline conditions. *Ann. Microbiol.,* 63: 225–232

Nadeem, S.M., Z.A. Zahir, M. Naveed, H.N. Asghar and M. Arshad, 2010. Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. *Soil Sci. Soc. Amer. J.,* 74: 533–542

Nadeem, S.M., Z.A. Zahir, M. Naveed, M. Arshad and S.M. Shahzad, 2006b. Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress. *Soil Environ.,* 25: 78–84

NARC, 2006. Golden Jubilee Report, NARC, Islamabad, Pakistan

National Centre of Organic Farming (NCOF) Annual Report, 2008. HQ of National Project on Organic Farming, Kamla Nehru Nagar, Ghaziabad, India

Naveed, M., M. Khalid, D.L. Jones, R. Ahmad and Z.A. Zahir, 2008b. Relative efficacy of *Pseudomonas* spp., containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of organic fertilizer. *Pak. J. Bot.,* 40: 1243–1251

Naveed, M., Z.A. Zahir, M. Khalid, H.N. Asghar, M.J. Akhtar and M. Arshad, 2008a. Rhizobacteria containing ACC-deaminase for improving growth and yield of wheat under fertilized conditions. *Pak, J. Bot.,* 40: 1231–1241

NFDC, 2005. Economic Survey of Pakistan 2005-2006. *Government of Pakistan, Finance Division*. Economic Adviser Wing, Islamabad

NFDC, 2008. *Pakistan Fertilizer Related Statistics*, p: 175. National Fertilizer Development Center, Planning Commission, Govt. of Pakistan, Islamabad, Pakistan

NIBGE Activity Report, 1998. Annual activity report of National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiology,* 17: 362–370

Qureshi, M.A., H. Shahzad, Z. Imran, M. Mushtaq, N. Akhtar, M.A. Ali and F. Mujeeb, 2013. Potential of rhizobium species to enhance growth and fodder yield of maize in the presence and absence of L-tryptophan. *J. Anim. Plant Sci.,* 23: 1448–1454

Qureshi, M.A., M.A. Shakir, M. Naveed, M.J. Ahmad, 2009b. Growth and yield response of chickpea to co-inoculation with *Mesorhizobium ciceri* and *Bacillus megaterium. J. Anim. Plant Sci.,* 19: 205–211

Qureshi, M.A., M.J. Ahmad, M. Naveed, A. Iqbal, N. Akhtar and K.H. Niazi, 2009a. Co-inoculation with *Mesorhizobium ciceri* and *Azotobacter chroococcum* for improving growth, nodulation and yield of chickpea (*Cicer arietinum* L.). *Soil Environ.,* 28: 124–129

Reddy, C.A. and R.S. Saravanan, 2013. Polymicrobial multi-functional approach for enhancement of crop productivity. *In*: *Advances in Applied Microbiology*, pp: 53–114. Sariaslani, S. and G.M. Gadd, (eds.), Academic Press, Elsevier Inc., New York, USA

Reed, M.L.E. and B.R. Glick, 2013. Application of plant growth-promoting bacteria for plant and soil systems *In*: *Application of Microbial Engineering*, pp. 181–228. Gupta, V.K., M. Schmoll, M. Maki, M. Tuohy and M.A. Mazutti (eds.). CRC Press Inc., New York, USA

Saleem, M., M. Arshad, S. Hussain and A.S. Bhatti, 2007. Perspectives of plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase in stress agriculture. *J. Indust. Microbiol. Biotechnol.,* 34: 635–648

Shaharoona, B., G.M. Jamro, Z.A. Zahir, M. Arshad and K.S. Memon, 2007. Effectiveness of various *Pseudomonas* spp. and *Burkholderia caryophylli* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.). *J. Microbiol. Biotechnol.,* 17: 1300–1307

Shaharoona, B., M. Arshad, and Z.A. Zahir, 2006a. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett. Appl. Microbiol.,* 42: 155–159

Shaharoona, B., M. Arshad, Z.A. Zahir and A. Khalid, 2006b. Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biol. Biochem.,* 38: 2971–2975

Shaharoona, B., M. Naveed, M. Arshad and Z.A. Zahir. 2008. Fertilizer dependent efficiently of Pseudomonads containing ACC-deaminase for improving growth, yield and nutrient use efficiency of wheat (*Triticum aestivum* L.). *Appl. Microbiol. Biotechnol.,* 79: 147–155

Shahzad, M.S., A. Khalid, M. Arshad, M. Khalid and I. Mehboob, 2008. Integrated use of plant growth promoting bacteria and P-enriched compost for improving growth, yield and nodulation of chickpea. *Pak. J. Bot.,* 40: 1735–144

Shakir, M.A., 2008. Microbial production of phytohormones by PGPR and their effect on wheat and cotton crops. *Ph.D. Thesis*. Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Sheikh, L.I., S. Dawar, M.J. Zaki and A. Ghaffar, 2006. Efficacy of *Bacillus thuringensis* and *Rhizobium meliloti* wth nursery fertilizers in the control of root infecting fungi on mung bean and okra plants. *Pak. J. Bot.,* 38: 465–473

Siddique, T., B.C. Okeke, M. Arshad and W.T. Frankenberger Jr., 2003. Enrichment and isolation of endosulfan-degrading microorganisms. *J. Environ. Qual.,* 32: 47–54

Siddiqui, I.A., 2000. Use of growth-promoting bacteria in the control of root -knot nematode and root-infecting fungi of crop plants. *Ph.D. Thesis.* Department of Botany, University of the Karachi, Karachi, Pakistan

Siddiqui, I.A. and S.S. Shaukat, 2002. Resistance against the damping-off fungus *Rhizoctonia solani* systemically induced by the plant growth promoting rhizobacteria *Pseudomonas aeruginosa* (IE-6S+) and *P. fluorescens* (CHA0). *J. Phytopathol.,* 150: 500–506

Sperber, J.I., 1957. Solution of mineral phosphates by soil bacteria. *Nature,* 180: 994–995

Stalstorm, Y.A., 1903. Beitrag zur kennturs der ein-wisking sterilia and in ha hung botindlichen strolte amt dil torlichkeit der phosphorsen der tricalcium phosphate. *Zentralblatt fur Baketeriologie,* 11: 724–732

Talat, M., 2000. Genetic mechanism of heavy metal resistance of *Pseudomonas aeruginosa* Cmg103. *Ph.D Thesis*. Department of Genetics, University of the Karachi, Karachi, Pakistan

Tien, T.M., M.H. Gaskins and D.H. Hubbell, 1979. Plant growth substances produced by*Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum*). *Appl. Environ. Microbiol.,* 37: 1016–1024

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil,* 255: 571–586

Zafar-ul-Hye, M., 2008. Improving nodulation in lentil through co-inoculation with rhizobia and ACC-deaminase containing plant growth promoting rhizobacteria. *Ph.D. Thesis*. Institute of Soil Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Zahir, Z.A., A. Munir, H.N. Asghar, B. Shaharoona and M. Arshad, 2008. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J. Microbiol. Biotechnol.,* 18: 958–963

Zahir, Z.A., H.M. Yasin, M.A. Anjum, M. Naveed and M. Khalid, 2010b. L-Tryptophan application enhances the effectiveness of rhizobium inoculation for improving growth and yield of mung bean (*Vigna radiata* (L.) Wilczek). *Pak. J. Bot.,* 42: 1771–1780

Zahir, Z.A., H.N. Asghar, M.J. Akhtar and M. Arshad, 2005. Precursor (L-tryptophan)-inoculum (*Azotobacter*) interaction for improving yields and nitrogen uptake of maize. *J. Plant Nutr.,* 28: 805–817

Zahir, Z.A., M. Arshad and W.T. Frankenberger Jr., 2004. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Adv. Agron.,* 81: 97–168

Zahir, Z.A., M. Zafar-ul-Hye, S. Sajjad and M. Naveed, 2011. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC deaminase for coinoculation with *Rhizobium leguminosarum* to improve growth, nodulation, and yield of lentil. *Biol. Fertil. Soils,* 47: 457–465

Zahir, Z.A., M.K. Shah, M. Naveed and M.J. Akhter, 2010a. Substrate-dependent auxin production by *Rhizobium phaseoli* improves the growth and yield of *Vigna radiata* L. under salt stress conditions. *J. Microbiol. Biotechnol.,* 20: 1288–1294

Zahir, Z.A., S.A. Abbas, M. Khalid and M. Arshad, 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. *Pak. J. Biol. Sci.,* 3: 289–291

Zahir, Z.A., U. Ghani, M. Naveed, S.M. Nadeem and H.N. Asghar, 2009. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. *Arch. Microbiol.,* 191: 415–424

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