Plant Archives Vol. 20, Supplement 2, 2020 pp. 1374-1379 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

REVIEW ARTICLE
BIOFERTILIZER: AN ALTERNATIVE OF SYNTHETIC FERTILIZERS

Swapnil Rai and Nidhi Shukla
Amity Institute of Environmental Science, Amity University Madhya Pradesh, Gwalior, India - 474005
srai@gwa.amity.edu, nshukla@gwa.amity.edu

Abstract

India is one of the nations that have large geographical area under agriculture since time immemorial. Most of the population of India is dependent upon the agriculture and are indiscriminately using chemical fertilizer in agriculture without following any guidelines. These chemicals are causing soil pollution and water pollution which reduces the crop production and are also affecting the living being including humans. When the synthetic fertilizers applied in excess, get accumulated in soil and plant or washed away with the water during monsoon season results the contamination of water bodies. Now days, biofertilizers have identified as an alternative of chemical fertilizers as they don't affect the environment and human health. These are eco-friendly substances, causing no effects on soil properties and economic. The living cells of biofertilizers convert naturally occurring minerals in to usable nutrients for plants and enhance the crop production. This paper deals with significant biological agents which have potential of biofertilizer. In India there is a need of innovation; research and public awareness for biofertilizer which is also important for sustainable agriculture.
***Keywords* :** Biofertilizer, eco-friendly, pollution, health hazard

Introduction

In India, due to growing human population it is necessary to increase crop production and land productivity to fulfill their food requirements. It is estimated that up to 2020, total production required by country will become 321 million tones of food grains and for their proper growth nutrient requirement will become 28.8 million tones but only 21.6 million tone nutrient will be available from a deficit about 7.2 million tones. To increase crop production use of chemical fertilizers and plant protection materials is also increased. Increasing use of chemical fertilizers in agriculture make country self sufficient in food production but it pollute environment and cause slow deterioration of living beings (Saxena and Joshi, 2002a).

Plant requires essential nutrients like nitrogen (N), Phosphorus (P), potassium (K), and several other minerals for their growth and receives them from soil (Arya, 2000). Nitrogen and phosphate relationship is very important and shows a direct impact on productivity of soil (Hutchinson and Richards, 1921). The fertilizers containing NPK are applied in soil to improve the crop productivity, but it is not utilized by crops completely. The remaining fertilizer pollutes the soil and also pollutes the ground as well as surface water sources through percolation and surface run-off during monsoon period. There are some other nutrients also contaminate the water bodies and leads to Eutrophication. India is one of the agriculture dominated country, hence most of the population dependent on agriculture. As India also have a large number of peoples and stands second position in world in population with 1.25 billion as per census 2011. In India agriculture is the most important part of economic growth. There are urgent need to increase the crop production hence farmers are using large amount of chemical fertilizers without following standard guidelines. This indiscriminate use of synthetic fertilizers affects the soil properties and also causes water pollution through runoff in rainy season. The liberal use of chemical fertilizers especially in paddy fields may affect the growth inhabiting micro organisms (Bishara, 1978; Konar and Sarkar, 1983) These fertilizers, reach into water bodies, also affect fishes (Palanichamy, 1985) and other organisms. The extensive applications of synthetic fertilizers from last several years have not only caused soil degradation but also polluted the soil and water with health implications in population.

In agriculture, chemical fertilizers are used extensively but they are costly and also have various adverse effects on soils i.e. depletes water holding capacity, soil fertility and disparity in soil nutrients. Due to insufficient uptake of these fertilizers by plants results in the leaching away from soil. Hence, it is necessary to develop some low cost fertilizers which work without disturbing nature.

Biofertilizers are biological cell that have potential to fix atmospheric nitrogen in to nitrates. Biofertilizer is a mixture which contains living cells specially micro-organisms that help the root system with good seed germination by providing the nutrients to them. These living cells can also solubilise insoluble salts like phosphate and can produce fertilizing substance in soil (Mazid *et al*., 2014).

Microbial fertilizers/ Biofertilizers/Biomanure:

During 1950s to 1970s considerable number of nitrogen fixing bacteria were found to be associated with crop. Several soil microbiologists suggests that Nitrogen fixing bacteria associated with the plants may be the source of agronomically significant nitrogen inputs to the sugarcane crop in Brazil (Ray and Handerson, 2001). A number of micro-organisms (bacteria, fungi and algae) are considered as beneficial for agriculture and used as biofertilizers. Microbial consortia are inoculated in the field for the improvement and supply of nitrogen, phosphorus, potassium and other essential elements which are necessary for the proper growth of plants. Micro-organisms produce a range of extra cellular enzyme which has the potential to mediate utilization of organic sources of nitrogen and phosphorus in soil (Saxena and Joshi, 2002b).

Biofertilizers are supposed to be a safe alternative to chemical fertilizers to minimize the ecological disturbance. These are natural, organic, non pollutant, cheap products that are required in a small dose (Gahukar, 2005-06). Microorganisms can fix atmospheric nitrogen, and solubilize insoluble phosphates, produce growth promoting substances for plants e.g. vitamins and hormones (Yojana, 1992). Many workers have reported that the uses of biofertilizers are beneficial for soil, as well as for crops. It is the safest method to maintain soil fertility. Biofertilizers are also called as "Microbial inoculants" (Subha Rao, 1982) due to use of many nitrogen fixing bacteria and cyanobacteria. Microbial inoculants are considered as new feature of agricultural system that facilitate or enhance the microbial process in the soil. Common biofertilizers such as *Azotobacter*, *Azospirillum*, *Rhizobium*, blue green algae etc. are good nitrogen fixer and *Bacillus megatherium var phosphaticum*, *Aspergillus awamori*, *Penicillium digitatum* etc. are good phosphate solubilizers.

Nitrogen fixing organisms such as *Azotobacter*, *Azotomonas, Azotococcus*, *Biejerinckia*, *Cyanobacteria* (*Anabaena spp*.) can fix nitrogen under aerobic condition and can be utilized for nitrogen deficient soil, while some facultative anaerobes such as *Bacillus*, *Klebsiella*, *Rhodo pseudomonas* and some species of the anaerobic genus *Clostridia* fix nitrogen under anaerobic condition (Emerich and wall, 1985). Nitrogen fixation by nitrogen fixing microorganism is catalyzed by the enzyme 'nitrogenase' which is inhibited by free oxygen (Emerich and Wall, 1985). The reduction of atmospheric nitrogen to ammonia by the nitrogenase enzymes is expressed as follows:-

$$N_2 + 8H^+ + 6e^- \rightarrow 2NH_4^+$$

Microbes are effective in inducing plant growth as they secrets plant growth promoters (auxins, abscisic acid, gibberellic acid, cytokinins, ethylene) and affects seed germination and root growth (Ramarethinum *et al.* 2005). They also play considerable role in decomposition of organic materials and enrichment of compost.

Biofertilizers have 75% moisture and it could be applied to the field directly. Biofertilizers contained 3.5% - 4% nitrogen, 2% - 2.5% phosphorus and 1.5% potassium. In terms of N: P: K, it was found to be superior to farmyard manure and other type of manure (Mukhopadhyay, 2006).

Table: N: P: K composition of different field manure:

Manure type	Nitrogen%	Phosphorus As P_2O_5, %	Potassium as K_2O%
Farmyard manure	0.40	0.20	0.40
Urban compost	0.60	0.50	0.60
Green manure	0.600.70	0.100.20	1.25
Biofertilizer (50% day)	1.802.40	1.001.20	0.600.80

Biofertilizers can be produced in the anaerobic digester used for production of biogas as a co-product of biogas. It consists of remaining residual solids after draining bioliquid. Excess sludge can perhaps serve as a fertilizers additive due to nitrogen fixing capability (Kargi and Ozmihci, 2004).

At present, annual production of biofertilizers is estimated as around 7000 tones from nearly 70 units and expected consumption of biofertilizers is approximately 6000 tones. In India nearly 3.5 lakh small and large biogas units have been installed during 1981-1991 (Makhopadhyay, 2006). Now Central Government also provides some financial assistance for setting up biofertilizer units.

Biofertilizers are an alternative to the conventional approach as they have lower cost than the chemical fertilizers and when they are required in bulk can be generated at the farm itself hence; these are economically attractive for the farmers (Venkatramani, 1996). The extent of cultivation depends on the market type and its proximity to the processing facility. Transportation costs and distance to markets will affect product value and its potential use (Saxena and Joshi, 2002b).

Nitrogen fixing Bacteria

Rhizobia: - Legume plants have root nodules, where atmospheric nitrogen fixation is done by bacteria belonging to genera, *Rhizobium* (fast growing rhizobia), *Bradyshzodium* (slow growing rhizobia), *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium* collectively called as rhizobia (Jordan, 1984; Chimote and Kashyap, 2001).

Rhizobium is a free living, gram negative, non sporulating, aerobic, motile and rod shaped bacterium which occur in soil. It occurs in the roots of leguminous plants and forms nodules where it fixes nitrogen in the presence of leghaemoglobin. Leghaemoglobin promotes O_2 utilization in bacteroids and favours nitrogen fixation and converts atmospheric nitrogen into ammonia in presence of phosphorus and molybdenum (Gahukar, 2001). In the absence of leghaemoglobin nitrogen fixation can not takes place. When rhizobial culture is inoculated in field, pulse crops yield can be increased due to rhizobial symbiosis (Dubey, 2001). *Rhizobium* resides in roots of beans, grams, groundnut and soyabean etc. *Rhizobium* is a crop specific inocultant. It can fix 15-20 kg N/ha and increase crop yield upto 20%. Higher Mg content due to inoculation of *Rhizobium* was observed by Kumudha (2005). Bhaskar and Kashyap (2004) reported that wild type strain of *R. ciceri* 18-7 mutant M 126 and complemented mutant M126 (C_4) were characterized for symbiotic properties (viz., acetylene reduction assay, total nitrogen content, nodule number and fresh and dry weight of the injected plants) and nitrogenase activity.

Azorhizobium is a stem nodule forming bacteria and for fixes nitrogen symbionts of the stem nodule also produce large amount of IAA that promotes plant growth (Ghose and Basu 1997). They have isolated *Azorhizobium caulinodans* from the stem nodule of the leguminous emergent hydrophyte *Aeschynomene aspera* and their purpose of study was to check the ability of *Azorhizobium spp*. for EPS (extracellular polysaccharides) production for its importance in nodule symbiosis and in industry. EPS acts as determinants of host plant specificity and play a role in the first step of root hair infection in N_2 fixation (Olivares *et al*., 1984). Ghosh and Basu (2001) also reported the plant *Aeschynomene aspera* possess many sessile stem nodule containing high amount of IAA.

Bradyrhizobium is also reported us good nitrogen fixer by various workers. Mathew *et al.* (2003) reported that when Mucunna seed were applied with an effective isolate of *Bradyrhizobium* in rubber (*Hevea brasiliensis*) Plantation, prior to souring it increased plant growth and consequently plant biomass, reduction in the weed population and increased soil microbial population were reported. It was also reported that *Bradyrhizobium* strain inoculation causes improvement in total organic carbon, N_2, available phosphorus and potassium in the soil.

Diazotrophs

These are aerobic chemolithotrophs and anaerobic photoautotrophs. These are non nodule forming bacteria. They include members of the families:-

1-Azotobacteracae e.g. *Azotobacter*:

They are the free living aerobic, photoautotrophic, non-symbiotic bacteria. They secretes vitamin-B complex, gibberellins, napthalene, acetic acid and other substances (Jain, 1998 and Gohukar, 2001) that inhibit certain root pathogens and improves root growth and uptake of plant nutrients. It was also reported that Azotobacter inoculation is effective in soil only in the presence of a native *Azotobacter* population. It was also reported that *Azotobacter* inoculation is effective in soil only in the presence of a native *Azotobacter* population. It occurs in the roots of *Paspalum notatum* (tropical grasses) and other spp. of this genus or other genera (Dobereiner, 1970). It adds 15-93 Kg N/ha/annum on *P. notatum* roots (Dobereiner *et al*., 1973). *Azotobacter indicum* occurs in acidic soil in sugarcane plant roots. It can apply in cereals, millets, vegetables and flowers through seed, seedlings soil treatment.

2-Spirillaceae - e.g. *Azospirillum, Herbaspirillum*:

These are gram negative, free living, associative symbiotic and non-nodule forming, aerobic bacteria. *Azospirillum* is a wide spread bacterium, occurs in the roots of dicots and monocot plants i.e. corn, sorghum, wheat etc. (Tarrand *et al*., 1978 and Elmerich, 1984), and responsible for nitrogen fixation in association with several cereals (Balandreau, 1983 and Vose, 1983).

It is easy to culture and identify. *Azospirillum* is found to be very effective in increasing 10-15% yield of cereal crops and fixes N_2 upto 20-40% Kg/ha. Different *A. brasiliense* strains inoculation in the wheat seed causes increase in seed germination, plumule and radicle length (Tien *et al*., 1979; Gunasekaran and Purushothaman, 1980). *Azospirillum* inoculations cause plant growth. It may be due to either by nitrogenase activity or by its ability to produce plant growth promoters (Okon and Labandera – Gonzalez, 1994). Ray *et al.* (2004) have conducted a field experiment to assess the response of winter and autumn rice varieties to *Azospirillim brasiliense*, strain Sp7 and *Azospirillum lipoferum*, strain C_2 with and without inorganic nitrogen. They have observed that there was an increase in the grain yield of all the 4 varieties as well as dry matter. They have also reported that *Azospirillim brasiliense* (Sp7) inoculation gave higher % grain and straw yield than *A. Lipoferum* (C_2)

Herbaspirillum species occurs in roots, stems and leaves of sugarcane and rice. They produce growth promoters (IAA, Gibberillins, Cytokinins) That promotes root development and enhances uptake of plant nutrients (N, P, K). In legume plants, *Azospirillum* fixes atmospheric nitrogen.

3-Acetobacter diazotrophicus:

Another diazotroph is *Acetobacter diazotrophicus* occurs in roots, stem and leaves of sugarcane and sugar beat crops as nitrogen fixer and applied through soil treatment. It also produces growth promoters e.g. IAA. That helps in nutrients uptake, seed germination, and root growth. This bacterium fixes nitrogen upto 15kg /ha/year and enhance upto 0.5–1% crop yield (Gahukar, 2005-06). It releases various organic acids (Mahesh Kumar, 1999), succinate, tarterate, citrate and gluconate etc. which generated H^+ion that dissolve mineral phosphate and make it easily available to plants. Mowade and Bhattacharya (2000) have reported that organism have the capacity to solubilising insoluble phosphate in Pikovskay's medium and Sperber's medium and tested for its response to different antibiosis under in vitro condition.

Cyanobacteria (Blue green algae)

Nostoc, *Anabaena*, *Oscillatoria*, *Aulosira, Lyngbya* etc. are the prokaryotic organisms and phototropic in nature. They play an important role in enriching paddy field soil by fixing atmospheric nitrogen and supply vitamin B complex and growth promoting substance (Sharma, 1986) which makes the plant grow vigorously. In paddy field there is no need of cyanobacterial inoculation. They also convert insoluble phosphorus into soluble form by excreting organic acids. Cyanobacteria fixes 20-30 Kg/N/ha and increase10-15% crop yield when applied at 10 Kg/ha. Cyanobacteria oxygenate the water impounded in the field. The cyanobacterial mat in paddy fields also reduces loss of moisture from the soil. Blue green algae can be used in the form of flakes or these flakes can be powdered and mixed with farmyard manure and soil then can be applied in the field. Algal flakes are dried and mixed at the rate of 10^{15} Kg/ha, after one week rice is planted (Arya, 2000).

Azolla-Anabaena symbiosis

It is a free floating, aquatic fern found on water surface having a cyanobacterial symbiont *Anabaena azollae* (hetero cystous) in their leaves. Azolla fixes atmospheric nitrogen in association with nitrogen fixing cyanobacteria in paddy field and excrete organic nitrogen in water during its growth and also immediately upon trampling. Azolla is used as biofertilizer in India, USA, Srilanka, China, Indonesia, Bangladesh and many other countries. Azolla fixes 40-60 Kg N/ha in a month and increases 10-20% yield of paddy crops (Kumar, 2004).

Azolla contributes nitrogen, phosphorus (15-20 Kg/ha/month), potassium (20-25 kg/ha/month) and organic carbon etc. and when applied in rice field also suppresses weed growth. It is also susceptible to high temperature (>40^0C) and scarcity of water (Gahukar, 2005-06). Azolla also absorbs traces of potassium from irrigation water. Azolla can be used as green manure before rice planting. Azolla spp. are metal tolerant hence, can be applied near heavy metal polluted areas.

Phosphate Solubilising Bacteria

Pseudomonas fluorescens, *Bacillus megatherium var. phosphaticum*, *Acrobacter acrogens*, *Nitrobacter spp*., *Escherichia freundii*, *Serratia spp*., *Pseudomonas striata*, *Bacillus polymyxa* are the bacteria have phosphate solubilising ability.

'Phosphobacterin' are the bacterial fertilizers containing cells of *Bacillus megatherium var. phosphaticum* prepared firstly by USSR scientists. They increased about 10 to 20 % crop yield (Cooper, 1959).

It can be applied for low land and upland rice. They dissolve soil phosphate and make it available for crops. A large number of soil microorganisms have the ability to solubilize inorganic phosphates through their metabolic activity directly or indirectly (Arya, 2000). They produces plant growth promoting hormones e.g. IAA, GA and various organic acids e.g. lactic acid, citric acid, fumaric acid, succinic acid etc. These organic acids help in phosphate solubilising activity of soil.

Phosphate solubilizing fungi

Some fungi also have phosphate dissolving ability e.g. *Aspergillus niger*, *Aspergillus awamori*, *Penicillium digitatum* etc.

Plant Growth Promoting Rhizobacteria (PGPR)

Recently ability of plant growth promoting rhizobacteria is also studies for microbial control. They are also called as microbial pesticides e.g. *Bacillus spp.* and Pseudomonas fluorescens. Sipirin (2000) reported that the growth and survival of vetiver is possible without nitrogen and phosphorus application especially in the infertile soil with the help of diazotrophs including the genera of *Pseudomonas*. Chakraborti *et al.* (2003) reported that four bacterial spp. *Serratia marcescens* TR10, *Ochrobactrum ocnthropi* TR9, *Bacillus pumilus* TR24 and Bacillus spp. TR16. isolated from rhizosphere of tea plant have antagonistic ability against the root pathogens of the tea plant. They have also reported that *Srratia spp.* and *Ochrobactrum spp.* are able to promote growth of plants. It was founded by Paul *et al*. (2003) that *Pseudomonas fluorescens* application to the Black pepper rhizophere resulted in easy mobilisation of the essential nutrients in the rhizosphere microcosm and resulted in enhanced uptake of nutrients, which reflected in increased plant biomass.

A study was carried out by Joseph *et al.,* (2003) in the Kerala from 10 rubber growing areas and isolated 17 fluorescent rhizobacterial strains and founded that all the 17 isolates shows antagonism against 5 pathogens of rubber. They also observed that these organism also improve the growth of *H.brasiliensis* and associated crop cover i.e. shoot weight, root weight, nodulation, nitrogenase activity.

Mycorrhiza

Mycorrhizas are developed due to the symbiosis between some specific root inhabiting fungi and plant roots. This means these are fungi grow on roots of the plants. There are specific fungi for vegetables, fodder crops, flowers, trees etc. Mycorrhizas are used as biofertilizers (Kumudha, 2005) and biocides. They absorb nutrients such as manganese, phosphorus, iron, sulphur, zinc etc. from the soil and pass it on to the plant. They also show higher tolerance to high soil temperature. Mycorrhizal fungi increase the yield of crop fields by 30-40%. Mycorrhiza also produces plant growth promoting substances. It was also reported that seedlings having mycorrhizal fungi growing on their roots grow faster after inoculation. Mycorrhiza occurs in low land and upland rice and it mobilize phosphorus required by rice.

Commonly mycorrhizas are of two types: Ectomycorrhiza and Endomycorrhiza. Both are different in structure and systematic position of fungi.

Ectomycorrhiza: They occur in the roots of the higher plants e.g. gymnosperms and angiosperms. These are the fungi requires pH 5-6 for their proper growth. Excessive use of inorganic fertilizers and shading supresses the development of ectomycorrhizal fungi. The poor development of mycorrhizal fungi in the roots of plants causes stunted growth and chlorosis of leaves.

Endomycorrhiza (Vam fungi):

They occur commonly in the roots of crop plants. These fungi penetrate the cortical cells and get established them intracellularly by secreting extracellular enzymes. VAM fungal hyphae enhance the uptake of phosphorus and other nutrients that is aresponsible for plant growth stimulation including roots and shoot length. (Hayman, 1980). VAM also enhances the growth of black pepper, protects from *Phytophthora capsici, Radopholus similis* and *Melvidogyne incognita* (Anandraj *et al*., 2001). It was reported by Kumudha (2005) that VAM inoculation was found to be effective in chlorophyll pigmentation. Trappe and Fogel (1977) reported that the increased nitrogen uptake in VAM might also be due to the increased phosphorus uptake which in turn might enhances the activity of NAD dependent enzyme which might contribute to nitrate reductase activity. VAM fungi enhance water uptake in plants and also provide heavy metals tolerance to plants.

Benefits from Biofertilizers:

They are environmentally sound, pollution free and of low cost. They increase crop yield upto 10-40% and fix nitrogen upto 40-50 Kg. After using 3-4 years continuously, there is no need of application of biofertilizers because parental inoculums are sufficient for growth and multiplication. They improve soil texture, pH, and other properties of soil. They produces plant growth promoting substances e.g. IAA amino acids, vitamins etc.

Precautions:

1- They are living organism hence, handling should be careful.
2- Biofertilizers should be used before expiry date.
3- They are species specific hence, particular biofertilizer should be used for a particular crop plant.
4- Recommended dose of biofertilizer should be applied.
5- They should not be exposed to direct sunlight.

References

Arya, A. (2000). Biofertilizer for sustainable plant development. *Everyman's Science*, xxxv (1): 19-25.

Anandraj, M.; Venugopal, M.N.; Veena, S.S.; Kumar, A. and Sarma, Y.R. (2001). Eco-friendly management of disease of spices. Indian species. 38(3): 28-31.

Bhaskar, V.V. and Kashyap, L.R. (2004), Azide resistance in *Rhizobium ciceri* linked with superior symbiotic nitrogen fixation. Ind. J. Exp. Biol. 42: 1177-2285.

Bishara, N.F. (1978). Fertilizing fish ponds II growth of *Mugil cephla* in Egypt by pond fertilization and feeding. Aquaculture. 13: 361-367.

Bishara, N.F. (1979). Fertilizing fish ponds III growth of *Mugil apito* in Egypt by pond fertilization and feeding. Aquaculture. 16: 47-55.

Balandreau, J. (1983), Microbiology of the association. Can. J. Microbiol., 29: 851-859.

Chakraborty, V.; Chakraborty, B.N.; Roy Chawdhary, N.; Tongden, C. and Basnet, M. (2003): Investigations on plant growth promoting rhizobacteria of tea rhizosphere. 6th International PGPR workshop, 5-10 October, Calicut, India.

Chimote, V. and Kashyap, L.R. (2001). Lipochito oligosaccharides and legume rhizobium symbiosis – A new concept. Indian J. Exp. Biol., 39: 401-409.

Cooper (1959). Soil fertilizers. 22: 227 – 233.

Dobereiner, J. (1970). Zent. Bakteriol II. 124: 224-230.

Dobereiner, J.; Day, J.M. and Dart, P.J. (1973). Perg. Agr Pe. Bras 8: 153-157.

Dubey, R.C. (2001). In: A text book of biotechnology, S. Chand & company Ltd. New Delhi.

Elmerich, C. (1984). Biotechnology, 2: 967-978.

Emerich, D.W. and Wall, J.D. (1985). In: Nitrogen fixation In: Moo-young, M. (Ed.), comprehensive Biotechnology, Vol. 4 Pergamon Press, NY, 73-106.

Gahukar, R.T. (2001). Kisan world, 28(3): 25-27.

Gahukar, R.T. (2005-06). Potential and use of bio-fertilizers in India Everyman's Science, XL(5): 354-361.

Ghosh, A.C. and Basu, P.S. (1997). Culture growth and IAA production by a microbial diazotropic symbiont of stem-nodule of the legume *Aeschynomene aspera*. Folia. Microbiol. 42: 595.

Ghosh, A.C. and Basu, P.S. (2001). Extracellular polysaccharide production by *Azorhizobium caulinodans* from stem nodules of Leguminous emergent hydrophyte, *Aeschynomene aspera*, Ind. J. Exp. Biol., 39: 155-159.

Gunasekaran, S. and Purushothaman, D. (1980). Nitrogen fixation by Azopirillum in the rhizophere of cotton. National symposium on Biological nitrogen fixation in relation to crop production. T.N. Agric Univ. Coimbtore, 27.

Hayman, D.S. (1980). Mycorrhiza and crop production. Nature, London, 287: 487-488.

Hutchinson, H.B. and Richards, E.H. (1921). J. Ministry of Agriculture, 28: 398.

Jain, R. (1998). Millenium Biofertilizer Guide, Nargi Printer, New Delhi. 52.

Jordan, D.C. (1984). Bergeys manual of sytemativ Bactercology, 234.

Joseph, K.; Sunju, V.; George, J.; Mathew, J. and Kunivilla, J.C. (2003). Plant growth promoting Rhizobacteria in Rubber (*Hevea brasiliensis*) plantations. 6th International PGPR workshop, 5-10 October, Calicut, India.

Konar, S.K. and Sarkar, S.K. (1983). Acute toxicity of agricultural fertilizers to fish. Geobios, 10: 6-9.

Kargi, F. and Ozmihci, S. (2004). Batch biological treatment of nitrogen deficient synthetic waste water using Azotobacter supplemented activated sludge. Bioresource Technology, 94: 113-117.

Kumar, N. (2004). Indian farmer's Digest, 37(2): 7-9.

Kumudha, P. (2005). Studies on the effect of biofertilizers on the germination of *Acacia nilotica* Linn. Seeds Ad. Plant Sci., 18(II): 679-684.

Mahesh Kumar, K.S. (1999). Curr. Sci., 76: 874-875.

Mathew, J.; Joseph, K.; Lakshmanan, R.; Jose, G.; Kethandaraman, R. and Kuruvilla, J.C. (2003). Effect of Bradyrhizobium inoculation of Mucuna Bracteala and its impact on the properties of soil under Hevea. 6th International PGPR workshop, 5-10 October, Calicut, India.

Mazid, M. and Khan, T.A. (2014): Future of Bio-fertilizers in Indian Agriculture: An Overview, International Journal of Agricultural and Food Research, 3: 10-23.

Mowade, S. and Bhattacharya, P. (2000). Resistance of P-solubilosing Acetobacter diazotrophicus to antibiotics, Curr. Sci.,79 (II).

Mukhopadhyay, S.N. (2006). Eco-friendly products through process biotechnology in the provision of biotechnology economy-Recent advances. *Technorama*, A. Supplement to IEI News, March.

Okon, Y. and Labandera-Gonzalez, C.A. (1994). Agronomic application of Azospirillum: An evaluation of 20 years worldwide field inoculation. Soil.Biol. Biochem. 26: 1591-1601.

Olivares, J.; Bedman, E.J. and Martinez, M.E. (1984). Infertility of *Rhizobium melilotias* affected by extracellular polysaccharides. J. Appl. Bacteriol., 56: 389.

Palanichamy, S.; Seeniammal, K. and Arunachalam, S. (1985). Effects of agricultural fertilizers complex on food consumption and growth in the fish *Sarotherodon mossambicum* (Trewaves), J. Env. Biol., 6(2): 71-76.

Paul, D.; Srinivasan, V.; Anandraj, M. and Sarma, Y.R. (2003). *Pseudomonas fluorescens* mediated nutrient flux in the black pepper Rhizosphere microcosm and enhanced plant growth. 6th International PGPR workshop, 5-10 October, Calicut, India.

Ramarethinum, S.; Murugusen, N.V. and Rajalakshmi, N. (2005). Pestology, 20(4): 12-14.

Ray, N.; Baruah, R. and Goswami, S.R. (2004). Effect of seedling bacterization of wetland rice with Azospirillum. Ind. J. Environ. & Ecoplan., 8(3): 843-848.

Ray, R.N. and Handerson, G. (2001). Endophytic nitrogen fixation in sugarcane: Present knowledge and future applications, Technical expert meeting on increasing the use of biological nitrogen fixation (BNF) in agriculture, FAO, Rome.

Saxena, P. and Joshi, N. (2002a). A comparative study of compost formation by the *Mucor sps.* and *Penicillium sps* on the soil. Him. J. Env. Zool., 16(1): 83-96.

Saxena, P. and Joshi, N. (2002b). In: Role of Microorganisms in the decomposition of organic wastes matter. Ph. D.Thesis, Gurukul Kangri University, Haridwar.

Sipirin, S.; Thinathorn, A.; Pintarak, A. and Aibcharoen, P. (2000). Effect of associative nitrogen fixing bacterial inoculation on growth of vetiver grass. A poster paper presented at ICV-2.

Subha Rao, N.S. (1982). In: Biofertilizers, In: Advances in agriculture microbiology (Ed.).Oxford and IBH Publication New Delhi. 219-242.

Sharma, V.K. (1986). A review of recent work on pesticide studies on the nitrogen fixing algae. J. Env. Biol. 7(3): 171-176.

Tarrand, J.J.; Kreig, N.R. and Dobereiner, J. (1978). A taxonomic study of the spirillum lipoferum groups, with description of a new genus, *Azospirillum gen. nov*. and two sps. *Azosperillum brasiliense sp nov*. Can J. Microbiol. 24: 967-980.

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

Trappe, J.M. and Fogel, R.S. (1977). In: Ecosystem function of mycorrhizae in the below ground ecosystem. A synthesis of plant associated process (Ed.) J.K. Marshall, Colarado-state University. Range, Sci, Deptt. Sci., services, No. 26 furt collins.

Venkatramani, G. (1996). In: Hindus Servey of Indian Agricultre, 29.

Vose, P.B. (1983). Development in non legume N2 fixing systems. Can. J. Microbiol. 29: 837-850.

Yojana, (1992). In: Biofertilizers, In:Advances in agricultural microbiology (Ed., Subha Rao N.S.) Pp 219-242, Oxford & IBH Pub. Co., New Delhi.