CHAPTER - I

INTRODUCTION AND REVIEW OF LITERATURE
INTRODUCTION

Biofertilizers are natural and organic products. They help to provide and keep in the soil all the materials and micro-organisms required for the plant growth. Their application is easy, cost-effective and does not cause pollution. In India, biofertilizers constitute the best renewable source of nutrient supply to plants and as supplement to chemical fertilizers and organic manures. However, there is inconsistent response and poor adoption by farmers. Therefore, high degree of motivation and innovative ability of the farmers are needed to exploit the potential of biofertilizer for sustainable agriculture and long-term benefits.

In India, there is constant pressure on crop production from the available cultivable land in order to keep pace with the food requirement for an ever-increasing population. Intensive agricultural practices exploiting the land and other natural resources increased manifold during the last 50 years. Simultaneously the use of chemicals as fertilizers and plant protection materials also increased. For example, consumption of nitrogen (N), phosphorous (P) and potassium (K) fertilizers increased over eight-fold and application dose about six-fold from 1971 to 1999. The cost of chemical fertilizers has also increased tremendously.
In intensive agriculture, chemical fertilizers are recommended and used by farmers sometimes unjudiciously, expecting to harvest high yields leading to a high degree of profitability. However, wrong and untimely applications of synthetic fertilizers adversely affect the natural balance of the soil/crop ecosystems, microbial ecology and environment, resulting in widespread decline in the crop nutrients in a low-fertility situation accompanied by the high cost of inorganic nutrients and the concern about environmental degradation and pollution, the need for supplementary cheaper sources of nutrients is recognized, and there is an increasing demand for organic food. This demand can be fulfilled by integrated nutrient management and adoption of new technologies including biofertilizer, among others, which are also vital components of sustainable agriculture. Therefore, there is an upward trend in the requirement of biofertilizers. Recently, Kakde et al. (2005) described the present status of supply, demand, marketing strategies and network, and government interventions in the pricing policies of biofertilizers in India.

In the field, crop response to the biofertilizers is not as spontaneous as farmers would experience with chemical fertilizers, because the former varies with the agroclimatic situations. There is lack of marketing infrastructure and distributing network; farmers are ignorant about biofertilizers; the companies of chemical fertilizers do advertise; have public support and assure higher benefits for retailers. Therefore, recommendations on biofertilizers need to be properly formulated considering the effectiveness in target crops and economics of such application (Gahukar, 2006).
Biofertilizers are latent or living cells of effective microorganisms (bacteria, fungi) which mobilize and augment the availability of plant nutrients such as by encouraging fixing of atmospheric N, solubilization and mineralization of P and K (Singh and Shukla, 2004). The term “microbial inoculant” is also used after the name of microorganism. Biofertilizers are natural, organic, non-pollutant, cheap products that are required in a small dose. According to Chandra and Kumar (2005) and Jat and Ahlawat (2004) residual effect of legumes on succeeding crop can result in an additional reserve of 20-60 kg N/ha. The physical, chemical and biological properties of soil are improved. Microorganisms secrete certain organic substances such as auxins, gibberellins, cytokinins, ethylene and abscisic acid that function as plant growth regulators and influence physiological processes resulting in better seed germination and root growth (Ramarethniam, et al. 2005). Natural resistance in plant builds-up against pests and soil-borne diseases, because antibodies are produced and beneficial microorganisms proliferate in the soil. Thus, biofertilizers can act as a renewable supplement to chemical fertilizers and organic manures (Singh et al. 2003).

Biofertilizers can be used for (i) treating the seeds before sowing by using adhesives, (ii) dipping of the seedlings in biofertilizer solution before replanting/transplanting and (iii) treating the soil by mixing with farm yard manure (FYM) during plant development.

For seed treatment with *Rhizobium, Azospirillum*, phosphorus-solubilizing bacteria (PSB) or *Azotobacter*, 50 g of crude sugar is dissolved in 500 ml of distilled water and the mixture is heated for 15 min. or 200g of gum
arabic is added as sticker. This mixture is left for cooling and 200 g of the inoculant is poured into it to form slurry. 10 kg of seed is mixed uniformly with slurry to get homogenous coating. These treated seeds are dried in shade for 10-15 min or used immediately for sowing, preferably during morning or evening hours. If synthetic fungicide is to be applied on seed, a time gap of 24 hrs. is necessary to avoid harmful effects on biofertilizer (Gahukar, 2001 and Jain, 1998). Generally, 20 g inoculant/ha is required for 1 kg of small-sized seed, whereas for medium-size seed (mung bean, cowpea, pigeon pea, lentil) and large-size seed (chick pea), the dose of 30 g and 40 g, respectively, is needed (Chandra and Kumar, 2005).

According to Chandra and Kumar (2005) Gahukar (2001) and Jain (1998) for dipping the seedlings of paddy or vegetables, 1-2 kg of biofertilizer is poured into 15 lit of water or 250 g inoculum of vesicular-arbuscular mycorrhiza (VAM) is poured in a liter of cowdung to form a slurry. Roots of seedlings from 10-15 kg seed, are dipped in this slurry for 10-30 min, followed by transplanting of the seedlings. In case of sugarcane, setts are dipped for 5-10 min in a suspension prepared by dissolving 1-2 kg of biofertilizer in 50-100 lit of water.

According to Gahukar (2006), for soil treatment, 500-800 g of biofertilizer is mixed homogeneously with 10-15 kg of FYM of compost, and this mixture is added to soil at the time of sowing or 24 hrs earlier or during interculturating, because biofertilizers require organic matter for their growth and development, as well as for their activity in soil. In case of blue green algae (BGA), dried flakes @ 10 kg/ha are applied 10 days after transplanting of
paddy when there is 2-3 cm standing water. *Azolla* can be used as green manure or as dual crop. For green manuring, *Azolla* is sown in the field or in a separate shallow pond. Water is drained off the field and *Azolla* is incorporated into soil before transplanting of paddy, @ 3-4 t/ha, directly or with the help of weeder. Dried inoculum of *Azolla* is also presoaked in 50 ppm of superphosphate solution for 12 hrs and inoculated in paddy field @ 2-5 kg/ha, a week after transplanting (Jain, 1998). As a dual crop, *Azolla* is intercropped with paddy and then mixed in the soil while main crop is growing (Chandra and Kumar 2005). The VAM is applied in nursery 3-5 cm below the seed. If seedlings are raised on seedbeds, about 5 kg inoculum is broadcasted over an area of 25 sq m after mixing with soil and is covered with a thin layer of soil (Jain, 1998).

The phototropic prokaryote bacteria, (Blue Green Algae (BGA) or cyanobacteria) are effective, only in submerged paddy in presence of bright sunlight by forming a bluish-green mat on standing water and by converting insoluble phosphorus into soluble form, fixing N to the tune of 20-30 kg/ha, thereby raising the crop yield by 10-15% when applied at 10 kg/ha. Biomass in the soil increases, organic compounds are liberated and plant growth is regulated. If high doses of chemical fertilizers are applied in a hot climate during crop season, the efficiency of algae is adversely affected to a great extent. Another difficulty is that establishment of inoculated species is difficult because of competition with grazers and green algae (Singh and Shukla, 2004).

*Azolla* is a free floating symbiotic fern found on water surface, individually or in mats, in lowland fields and water bodies. This fern fixes
atmospheric nitrogen in association with nitrogen fixing BGA, *Anabaena azollae* in flooded paddy. Another common species, *Azolla pinnata* grows in 7-12 days and decomposes readily in soil, rendering nitrogen available to plants in a short time. When it is used as green manure, weeds are suppressed to some extent due to large quantity of biomass as *Azolla* is capable of growing profusely in shallow water system. One kg of *Azolla* fixes 40-60 kg N/ha, 15-20 kg P/ha and 20-25 kg K/ha in a month, thereby increasing yield of flooded paddy by 10-20% (Kumar, 2004). However, *Azolla* is susceptible to high temperature (>40°C) and scarcity of water, and needs phosphorus in large quantity for being effective. Maintenance of *Azolla* culture in pond is difficult during winter (Chandra and Kumar (2005). About bio fertilizers the facts were considered as under:

1. Biofertilizers should be purchased from an authorized/recommended company shop or retailer. The date of manufacturing and expiry of utilization must be verified before delivery.

2. During storage, packets should not be exposed to direct sunlight and not to be stored with chemical fertilizers and pesticides.

3. In case of different seed treatments, seeds are to be treated first with fungicides, then with insecticides and lastly with biofertilizers.

4. For enhancement of a crop an appropriate and recommended dose of biofertilizer should be used.

Spurious and sub-standard biofertilizers are sold because there is no strict control over quality production. Further, ISI specifications have been formulated only for *Rhizobium, Azotobacter* and *Azospirillum*, and
location-specific strains are yet to be developed; facilities and regulatory acts for testing biofertilizers are meager, carrier material of long shelf life is not available. All these factors affect viability of formulations. In fact, packet is often sold even after expiry date or material is used for 2-3 applications after opening the packet.

The present expiry period is limited to 6 months which is related to carrier (lignite or charcoal). Storage at 20°C is recommended; wherever packets are stored at higher temperature (40°C), viability of inoculant is lost within few hours. While packing, the moisture content of the carrier should be >50% whereas plastic bags are filled in with material having less or more moisture that kills spores. Moreover, high moisture attracts contaminating micro-organisms that either compete with biofertilizer or have antagonistic interaction. Retail shops do not sell biofertilizers because of short shelf life, limited demand and lack of storage facilities. Transportation for distribution is a major problem in rural areas.

According to Gautam and Pant (2001) and Gill et al. (2000) lack of soil moisture or drought, water logging, unfavorable soil pH, high temperature, higher content of nitrates, deficiency of phosphorus, molybdenum, iron, copper, competition from native stains of inoculants and presence of toxic chemicals in soil; all of them reduce the viability of spores. Soil insects, snails, nematodes and harmful microorganisms can disturb the activity of biofertilizers. Nitrogen fixation in acidic or alkaline soils is affected by low pH and is often associated with aluminium and manganese toxicity and calcium deficiency. Other problems are associated with compatibility with host genotypes, improper handling, faulty inoculation techniques and improper
dose. Therefore, visible effects of biofertilizer application are not generally observed in traditional agriculture.

According to Brencic and Winans, (2005) the new techniques of application, particularly use of methyl cellulose (1%) for seed coating, and pellets for direct soil application, should be encouraged. Recently, microbial sensing and response mechanisms in the form of cell to cell communication through the use of small signalling molecules have been reported. In case of iron deficient soil, *Pseudomonas* spp. colonizes crop roots that result in improvement in plant growth (Ramarethinam, *et al.*, 1998 and Yeole *et al.*, 2001). These aspects are important in dry soil affecting bacterial population in rhizosphere. Pelleting of seed with lime for acidic soil and with gypsum for saline soil is beneficial. Chemical fixation in soil limits the use of biofertilizers, therefore, different strains of PSB mixed with rock phosphate and single super phosphate may be needed. If availability of phosphate is <10 ppm, P-fertilizers should be applied for N-fixing boifertilizer (Bagyaraj, 2003). Attempts ought to be made to explore the efficiency of suitable stabilizer/carrier with enough moisture (>40%) in extending the present expiry period of 6 months. Instead of legal acts, awareness campaigns including extension and field demonstrations would encourage farmers to use biofertilizers and make the crop cultivation ecofriendly and economically profitable. For making these campaigns effective, attention to the government agencies is needed towards training programmes particularly for the unemployed youth and progressive farmers. Local supply can be organized through network under the supervision of trained personnel. High degree of motivation, a firm commitment and innovative ability is however needed for successful utilization
of biofertilizers. Facilities for testing the contents and field effectiveness of commercial products need to be provided. Also, ISI specifications for liquid formulations are not available to production units (Raychaudhuri, 2004). Legislation for standard products concerning contaminant allowance, authenticity of strains, testing parameters for different formulations etc. seems to be important criterion for awareness and popularization. Random sampling at the level of manufacturer, trader and user may be introduced to verify stains, its efficiency and expected total viable spore counts.

According to Kumar et al. (2001); Alagawadi and Gaur (1998); Chendrayan et al. (2003) and Meshram et al. (2004) farmers should be advised to mix biofertilizers to get benefit of the synergistic effect on plant growth and ultimately on yield, but multistrain biofertilizers may be avoided. Further, compatibility of native stains with crop cultivar is another area for research. Use of biofertilizers as supplement of chemical fertilizers is beneficial particularly when N fertilizers are applied in split doses but not exceeding 40 kg N/ha at a time. If chemical fertilizers are to be applied to soil, there should be a gap of 15-20 days for better N-fixation (Singh et al. 2003). In storage, transportation or distribution, packets are exposed to sun rays, direct contact with chemicals, seed coating toxicity incompatible pesticidal or mineral additives etc. Cold storage facilities or safe storage in sheds should be provided at district/tehsil level to facilitate timely availability to farmers at the village level as majority of farmers use biofertilizers as and when needed and even after expiry date. Otherwise farmers should store packets in small earthen pots covered with a lid or cloth. These pots can then be kept in a pit covered by sand to maintain low temperature (Gahukar, 2006).
Man started cultivation on land in an organized way for food grains around 8000 B.C. Very soon he observed that if the same land is used for cultivation repeatedly the growth of plant is minimized upto a great extent and this led to the birth of many thoughts in his mind about the ways and means of improving the fertility of soil. The earliest records indicate that Romans and Aryans had many manuals for farmers to improve the cultivation of crops. For instance, columella’s treatise called “Husbandary” written about 60 A.D., contains descriptions of several agricultural practices which were used in the Roman empire for many generations. After the end of the Roman empire, the Arabian culture flourished in the 12th century and Moorish scholar, Ibn-al-Awan wrote an hand book on agriculture. In 18th century some farming practices came into existence, such as those of Jethro Tull (English)and the Norfolk “Four Course” system developed in Holland by years of experience based on crop rotation (Rao, 1986). Meanwhile, school of thoughts were given by many people regarding the ingredients in soil which nourish plants. In the 16th century, Bernard Pallissy, a potter to the French royalty gave his view that plant residues contained the ‘salt’ or the ‘principle’ which supported plant growth while in another view Jan Baptistavan Helmont believed that water was the principle capable of supporting the growth of plants. Later, by experiments of John Woodward of England this was established that certain ingredients in soil dissolved in water actually support plant growth. However, all these conjectures were set aside when a great finding came into light and this was from a French scientist, Antoine Lavoisier who developed a table of chemical elements in 1794. He also showed that plants and animals use up, oxygen by a process of respiration (U.S.D.A., 1957).
Fertilizer nitrogen will continue to serve for increasing grain production until a foreseeable future but efforts should also be made towards augmenting biological nitrogen fixation met by microorganisms. An average acre of gain legume like soyabean, beans of peas provide sufficient protein for 1000-2000 days for one person where as an average acre of plant materials converted to animal protein like beef and poultry provides only for 75-250 days (Hardy, 1974). Accurate estimates of annual turnover of nitrogen in the biosphere vary from 100 to 200 million tonnes (Delwiche, 1970; Burns and Hardy, 1975; Burris, 1977). The ratio between chemically fixed nitrogen and biologically fixed nitrogen ranges approximately from 1:4 to 1:2.5 and within biological fixation, and in the legume fixation, the amounts of kg N₂ fixed/ha/yr for individual legume has been estimated as follows: 125-135 for alfa-alfa, 85-190 for red clover, 80-150 for peas, 65-115 for soyabean, 65-130 for cowpea, and 90-155 for vetch (Alexander, 1977).

The world's population, food production and fertilizer consumption have increased gradually. It has been forecasted that there will be a further increase in population in the developing countries by about 2.1 million people at the end of 2010 A.D. To guarantee enough food to all, either the population growth has to be stemmed or more fertilizer has to be found to meet the ever increasing demand for protein. The increase in world fertilizer requirement by 2010 A.D. would be approximately three times the rate of current consumption.

The demand for chemically fixed nitrogen is bound to be on the increase and the nitrogen gap would be widened in incoming days. Such a gap
would be difficult to bridge in the wake of the energy crisis. Further more, in the field of chemical fixation, still no major break through is visible to cope up with the energy requirements of the conventional Haber-Bosch process for the production of ammonia. In India the construction of new nitrogen fertilizer plants is not only expensive but time consuming.

Nineteen seventies was the decade that witnessed escalating petroleum crisis, there by enormously increasing the organic fertilizer prices. To mitigate the problem, biologists came out with biological substitutes to organic fertilizers- the so called 'BIOFERTILIZERS'.

The term 'Biofertilizer' or which can be more appropriately called 'microbial inoculants', can be generally defined as the preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing, or cellulolyte microorganisms used for application to seed, soil or composting areas with the objective of increasing the members of such microorganisms and accelerate certain microbial processes to augment the extent of the availability of nutrients in a form which can be easily assimilated by plants. In a larger sense, the term may be used to include all organic resources (manures) for plant growth which are rendered in an available form, for plant absorption through microorganism or micro-organism plant associations or interactions. Such micro-biological processes may be as complex as that or nitrogenase mediated reactions in nitrogen-fixing micro-organisms which reduce elemental nitrogen into ammonia or as simple as the organic acid secretion by phosphate dissolving bacteria.

An ideal fertile soil is not only characterised by optimum physical
properties and chemical constituents conducive for plant growth, but also by microbiological processes are part of nitrogen, phosphorus and carbon cycles. In fact, wild eco-systems have sustained through centuries by means of such natural inter-conversions of essential elements (Rao, 1986).

The modern day intensive crop cultivation requires the use of chemical fertilizers, but fertilizers are not only in short supply but also are expensive in the developing countries. Therefore, the current trend to explore the possibilities of supplementing chemical fertilizers with organic ones, more particularly biofertilizers of microbial origin is of prime importance. In soil, myriad of micro organisms are at work in fixing nitrogen, mobilising other plant nutrients and degrading ligno-cellulosic wastes. Very often microorganisms are not as efficient in natural surroundings as one would expect that to be and therefore, artificially multiplied cultures of selected microorganisms play in accelerating natures way of recycling organic resources.

In addition to providing economy, their fast growing activity enriches the soil with nitrogen thus avoiding the need of crop rotation. Moreover the emission of pollutants from fertilizer industries could be considerably minimized, since there is no question of pollution in a biologically controlled ecosystem.

Nitrogen is a free commodity in the atmosphere. A positive balance of usable nitrogen on the earth depends upon nitrogen fixation which is a process by which atmospheric nitrogen \((N_2)\) is converted either by biological or chemical means to a form of nitrogen, such as ammonia \((NH_3)\) that can be used by plants and other biological agents. In biological nitrogen
fixation micro-organisms, either free living or in symbiosis with plants, reduce $N_2$ to NH$_3$ at atmospheric pressure and within the temperature range of 20° C to 30° C.

The idea of ‘inoculation was indirectly known to our ancestors when they transferred large amounts of soil from areas where leguminous crops were flourishing to areas, where they were less luxuriant. In a sense, they were ‘inoculating’ nodule forming bacteria from one field to another. With the advent of an energy crises, non-symbiotic nitrogen fixing micro-organisms got their due emphasis and applied research took shape in developing countries.

Biological nitrogen fixation and its importance to soil fertility have been known since the early 1800s.

Applied work relating to biofertilizers has been done in India on a large scale with legumes as well as non-legumes. Biofertilizer technology appears to be relevant to poor nations. Sophisticated basic research involving expertise and money, which can improve the ability of crops or micro-organisms to get the most out of microbiological processes is being increasingly carried out in U.S.A., U.K. and Australia. Studies on biological nitrogen fixation have thus become a multidisciplinary approach but microbiologists and agronomists are jointly involved in the application of biofertilizer technology at the field level.

Nitrogen-fixing bacteria, blue-green algae and aquatic *Azolla* containing nitrogen fixing blue green alga- *Anabaena azollae* were highlighted,
microbiological methods of energy production such as biogas and alcohol come to be recognized as the future hope of mankind.

In developing countries like India where there is immediate need to rely increasingly on organic fertilization of soil, these bio-fertilizer play a role in minimizing dependence on inorganic nitrogenous fertilizers. The biofertilizers, otherwise called microbial inoculants, are preparations containing live or latent cells of efficient strains of nitrogen fixing microorganisms used for seed or soil application. The main objective of applying inoculants is to increase the number of such micro-organisms in soils or rhizosphere and consequently improve the extent of micro-biologically fixed nitrogen to plant growth. Application of biofertilizers in combination with organic nitrogenous fertilizers has a key role to play in the economic management of nitrogen needs of crops.

The energy crisis and consequent increase in cost of nitrogen fertilizers, the widening gap between indigenous supply and demand of nitrogen and the low purchasing power of the cultivators have imposed serious limitation in rice production. Biological nitrogen fixation is being considered as an alternate at least in part, to chemical fertilizers in the global strategy for increasing the production of crop (Roy, 1984).

**REVIEW OF LITERATURE**

Bacteria and blue green algae play a significant role in meeting the nitrogen needs of crops. The occurrence of nitrogen fixing microorganisms such as *Azospirillum* within the plant parts of major economic plants and the symbiont *Anabaena azollae* within the equatorial fern, *Azolla,*
are being recently tried in Indian Agriculture. Man made inoculants are helping in the establishment of leguminous crops and also in increasing the production of protein rich food and fodder crops (Raju, 1980)

There are both novel and congenital approach to biological nitrogen fixation problem. Conventionally attempts should be made:

1. to increase the area under cultivation of grain legumes by the introduction of legumes in inter, multiple and relay cropping,
2. to provide efficient strains of Rhizobium for inoculating cereals,
3. to evolve a technology enabling the newly introduced nitrogen fixers to successfully compete with the strains of nitrogen fixing micro-organisms already present in soil,
4. to evolve nitrogen responsive (both biological and industrially fixed nitrogen) varieties of plants,
5. to overcome the inhibition of fertilizer nitrogen on biological fixation of nitrogen,
6. to define agronomic practices leading to better fixation and conservation of nitrogen on the farm,
7. to discover in-expensive nitrification inhibitors such as neemcake, and
8. to evolve simple practices to conserve water on the farm because optimum moisture is needed for successful nodulation and hence biological nitrogen fixation in legumes.

There is no limit of speculation in the unconventional area, which is indeed receiving considerable attention in recent years. Some of the considerations are:
1. to extend nitrogen fixation to cereal by inducing the formation of nodules on root,

2. to hybridize nitrogen fixing bacteria with non-nitrogen fixing bacteria by transformation, transduction and conjugation,

3. to implant nitrogen fixation capacity from nitrogen fixing bacteria into crop plants by protoplast fusion and tissue culture methods,

4. to overcome barriers in intergeneric hybridization between nodulating legumes and cereals, both at the floral level in intact plants and at the cellular level, in cell cultures,

5. to synthesize ‘nitrogenase’, the enzyme responsible for converting $N_2$ to $NH_3$ and harness it for use in nitrogen fixation as a catalyst in industrial processes, and

6. to extend research into new nitrogen-fixing association among plants and harness them to agriculture.

To overcome the hazardous effects of water logging and salinity on growth of crop plants, scientists in various laboratories are engaged to raise salt tolerant crop plants to over come of food shortage all over the world. But on the other hand in natural habitats like in land salt lakes, nature has already developed salt tolerant species of cyanobacterial and green algal forms which grow luxuriantly in the brine of these lakes. In land Sambhar and Didwana salt lakes, various species of blue green algae and green algae grow luxuriantly since earlier time (Gupta and Bajaj, 1983). The planktonic algae form a thick scum on the surface of salt Kyars and creates great problem in crystallization and extraction of salt. After heavy growth thick algal sheets
are scrapped manually form salt Kyars and allowed to dry in neaps on margins of Kyars. Since early times it has been a customary to auction these thick algal sheets or clumps to the villagers and they used them as fertilizer or manure in the sweet water irrigated crops like wheat, maize and barley (Gupta, 1972).

It has long been recognized that flooded rice soils maintain a degree of nitrogen fertility (Singh, 1961). Long term fertility trials have indicated that there is a major input of nitrogen into flooded rice soil. Much of this input attributed to $N_2$ – fixation by heterotrophic micro-organisms associated with rice roots, *Azolla*, blue green algae, and photosynthetic bacteria (Singh, 1961; Rodger and Kula sooriya, 1980; Rodger *et al.* 1985).

With the aim of the utilization of organic amendments or biofertilizers due to very high cost of chemical nutrients, the various attempts have been made with the hydrophytes or with the algae as biofertilizers.

Blue-green algae play a role in the nitrogen economy of tropical rice soils (Dee, 1939; Singh 1961). The nitrogen fixing algae can be cultured in open air tanks and used for rice cultivation. The results obtained by algal inoculation of rice fields in India have shown the possibility of using algae as a biofertilizer in rice cultivation

The blue green algae possess the twin abilities of photosynthesis as well as biological nitrogen fixation. Analysis of the blue-green algal flora from rice fields has revealed the occurrence of species of *Anabaena, Anabaenopsis, Aulosira, Cylindrospermum, Nostoc, Calothrix, Scytonema, Tolypothrix, Fischerella, Haplosiphon, Mastigocladus, Stiegonema, Westiella, Westiellopsis, Campylonema and Microchaete* as dominant nitrogen
fixers. Besides fixing nitrogen, these algae excrete vitamin B<sub>12</sub>, auxins and ascorbic acid which may also contribute to the growth of rice plants (Foggi, 1939; Dee, 1939; Singh, 1961; Stewart, 1970, 1971 and 1974).

Cheng et al. (1979), Kannaiyan et al. (1981), and Singh and Singh (1985) used *Azolla* as fertilizer and found improved rice growth. Likewise Rodgers et al. (1979) and Rao (1983) used blue green algae as manure and found increasing results. Ivanok and Dedyurina (1979) used straw and *Trigonella*. Broker and Guewara (1979) used tropical woody legumes, and Mohan and Katiyar (1981), used a number of organic amendment of plants, for the improvement of plants. Singh and Singh (1984) and Singh and Singh (1985) used *Azolla* along with blue green algae for better fertility of paddy soils.

Hupesh Institute of Hydrobiology (1971) reported that introduction of nitrogen fixing blue green algae into rice seedling leads to increased number of panicles/unit area, panicle length, average number of grain panicles and gave yield increase of 7.68% in early rice and 9.3% in late rice.

and Patil (1984), Ram and Rawat (1984), Sawabhe et al. (1985), Mandhare et al. (1990) and Pachapande (1990) for yield of paddy; Kaushik and Venkataraman (1979) for vitamin C; Latchumanan et al. (1979) for carbohydrate; Gulati (1990) for yield and protein content; Latchumanan et al. (1979) for yield, nitrogen, phosphorus and potassium content of plants, observed beneficial results.

Goyal and Roy (1996) reported that yield of rice increased with algal biofertilizers. It was observed by Gopalaswamy et al. (1997) that in rainy season, blue-green algae plus 50 kg N/ha increased yield of rice as compared with N alone. The grains yield of rice cv. Saket-4 was found highest with BGA followed by N alone (Pandey et al., 1993). Based on a field trial Kaushik (1994) showed that blue green algal biofertilizer gave higher grain yield for rice. Mohapatra and Chandrajee (1991) observed significant increase in grain yield from cyanobacteria inoculation.

Chandrasekaran et al. (1987) reported increase in grain yield on applying BGA biofertilizer. According to Gupta et al. (1987) cyanobacteria increased the crop yield of *Triticum aestivum, Zea mays, Brassica campestris, Vigna mungo, Pisum sativum and Cicer arietinum*. Blue green algae treated plots showed greater yield than bacteria inoculated plots (Gupta et al., 1989). Dubey and Rai (1995) found in a field trial that grain yield of paddy was increased with cyanobacteria. Gupta and Pandher (1996) presented a data on the effects of blue green algae as a biofertilizer on yield of several field crops.

Algal biofertilizer was recommended only as a supplement to nitrogenous fertilizers (Goyal, 1993). According to Roger et al. (1992) blue-green algae has potential to increase rice production.
Recently Pasricha et al. (1996) reviewed the utilization of organic manures including biofertilizer in relation to several field crops and Singh and Bisoyi (1993) reviewed on algal application in relation to soil fertility and crop plants specially rice plants. Palaniappan et al. (1995) reflected the benefits of organic manure including biofertilizer such as Azolla as well as blue-green algae on economics of nutrients.

According to Sompon et al. (1991) rice yield, receiving blue-green algae, increased. Badawy et al. (1996) observed that the growth, number of grains and grain yield of rice was improved by application of Azolla filiculoides. The increase in grain yield was equivalent to supplying 33 kg N/ha as urea. Dubey and Sharma (1995) were of the opinion that increased grain yield by cyanobacteria was equivalent to those obtained with 20 to 30 kg N/ha. The use of BGA biofertilizers increased the grain and straw yields and panicle number and N uptake by rice (Singh et al., 1988). An increase in nitrogen content in straw, grain yields and the protein content of rice was recorded by Antarikanonda et al. (1991) due to application of algal biofertilizers.

Cyanobacteria promote dry weight, total N and chlorophyll a and b and carotenoid of wheat plants (Abd Alla et al., 1994). In a field trial Singh et al. (1997) observed that the yield of rice and uptake of N, P and K by rice and wheat was increased by cyanobacteria as biofertilizer. Dry matter yield and total N content of the root, shoot and grain were significantly greater in plants inoculated with NH$_3$-excreting strain of Anabaena variabilis (Kamuru et al., 1998).

Grain yield of rice responded to increasing rates of urea. Inoculation of seedling roots with Azatobacter chroococcum or blue green algae increased grain
yield. Combined inoculation was superior to single (Rao et al., 1977).

A survey of nitrogen fixing blue green algae in rice fields of Sri Lanka was undertaken as a prelude to a wider research programme which envisages the exploitation of these organisms as biological fertilizers in rice cultivation. Pangrahi and Singh (1978) evaluated effect of ammonium sulphate, superphosphate, cytrolane on growth and nitrogen fixing potentiality of *Aulosira* spp. inoculated at the rate of 30 kg fresh material/ha. The grain yield was found to be increased by 107, 72, 105 and 85% over control respectively.

Biologically active compound may be liberated from blue green algae (*Anabaena* and *Nostoc*) growing on the surface of moist soil or may also be released as exudates from algae. Their inoculation in pots containing radish or tomato plants resulted in increased growth rate of plants and increased their over all yield (Rodgers, 1979). Kaushik and Venkataraman (1979), while working on tomato plants reported positive response to algal inoculation in terms of the yield of fruits and shoots, but there was no significant effect on vitamin 'C' content of fruits. A combined application of urea and algae was more effective than application of urea alone. Advancement in ripening of the tomato fruits by a week was also observed in the algalized series (Kaushik, 1979).

Application of 100 g:

(a) dried flakes of BGA, or
(b) peat based *Azotobacter*L water, or
(c) (a) +(b)
have increased the nitrogen, phosphorous and potassium contents in paddy plants at the tillering and maturation stages (Latchumanan, 1979).

On the other hand Latchumanan et al. (1979) reported that mixed culture of Azotobacter and blue green algae had no significant effect on carbohydrates content in rice grains.

Blue green algae’s biological activities can contribute 20-30 kg of biologically fixed $N_2$ per hectare. In addition they have been found to synthesize growth regulators like vitamin $B_{12}$ and auxin like substances. Use of blue green algae can result in saving of 20-30 kg N/ha. Besides fixing $N_2$ the blue green algae have natural advantages. Due to rapid growth under water logged condition, ability to withstand desiccation, high degree of adaptability, blue green algae production alone could become a rural vacation to generate additional income from the sale of blue green algae material (Goyal, 1982). Kannaiyan (1982) reported that blue green algae applied at rate of 10 kg/ha with levels of nitrogen resulted in increased grain yield. Similarly, Kannaiyan et al. (1982), observed increased grain yield in paddy due to application of blue green algae. All the treatments have recorded higher grain yield when compared to untreated ones.

According to Chandrakar et al. (1983) applications of 80 kg bga/ha to rice, grown at several locations increased the paddy yield by upto 19% and showed a residual effect on seed yield of Cicer arietinum. According to Rao (1983) grain yield/plant in Jaganath rice plants treated with algal mixture was 2.30 and in control 1.95 g.
In pot trials, soil inoculation with nitrogen fixing blue green algae (*Nostoc*) increased the paddy and straw yield of rice by 67% (Bongale, 1984). Paddy yield with *Azotobacter + 10-50 kg N/ha* was higher than with N alone. It was highest with blue green algae +75-100 kg N/ha (Bagal and patil, 1984). Bhuiya *et al.* (1984) was of the opinion that neither nitrogen application nor blue green algae inoculation affected 1000 grain wt. but with increased grain yield. A combination of 40 kg nitrogen and 10 kg blue green algae/ha gave highest grain yield which was 44% higher than the untreated i.e. control. Inoculation with blue green algae, 10 days after transplanting increased paddy yield even in absence of nitrogen (Sawabhe *et al.*, 1985).

Rao (1986), observed that increasing nitrogen rates significantly increased the yield components and yield. Inoculation either with *Azotobacter* or blue green algae significantly increased the yield. The effect of combined inoculation of these two inoculation on grain yield was additive. The beneficial effect observed with combined inoculation was equal to that obtained with 50 kg N/ha as urea.

The cost of the algal material when produced on the farmers field is negligible although commercially the cost for 10kg/ha of algae may come to Rs. 30/-. The saving on fertilizer nitrogen by this input is Rs. 100/ha if 25 kg N/ha is provided for rice cultivation by blue green algae. Recently Agnihotri *et al.* (2005), Mohan *et al.* (1989, 2003, 2004, 2005 and 2006) Singh *et al.* (2004) and Srivastva *et al.* (2003) observed significant increase in growth and composition of linseed plants with application of bga as biofertilizer.

The perusal of above literature reveals that considerable amount
of work has been done to the effect of biofertilizer on yield of paddy plants. However studies related with the effect of biofertilizer supply on growth and composition of plants are scarce.

Regarding the effect of biofertilizer, Mohan et al. (1987) found encouraging results on growth, ascorbic acid and chlorophyll content of tomato plants and with interactions of GA and biofertilizer they suggested that biofertilizer can be of practical significance.

**JUSTIFICATION OF THE PROBLEM**

Scanning of the literature reveals that, though, to some extent other aspects of the effect of biofertilizer (blue green algae) on plants have been studied, the data on the effect of biofertilizers on growth, metabolism and mineral composition of plants is neither sufficient nor well understood.

The dose and forms of biofertilizers have not been worked out sufficiently, in spite of the suggestions of Mohan (1983), and Mohan et al. (1987) that biofertilizers can prove valuable for solving many specific problems and the study of biofertilizers (blue green algae ) application may be of practical significance.

The energy crisis and consequent increase in the cost of chemical fertilizers, the widening gap between the indigenous supply and demand of nitrogen, and the low purchasing power of cultivators have imposed serious limitations in rice productions. Biological nitrogen fixation is being considered as an alternative, at least in part of chemical fertilizers in the global strategy for increasing production of crops.
The adoption of organic manure as compared to nitrogen fertilizer would be very less expensive, besides being quite safer as compared to the chemical fertilizers.

Besides understanding fundamental problems and altered effects on plant and metabolism by effect of biofertilizers, the proposed study would explore the possibilities of increasing yield of crop plants in favour of human welfare and can prove economically very useful for boosting the green revolution to feed the growing needs of increasing population, while on the other hand it would also prevent the need from disturbing the ecosystem.

Thus the present investigation is a meaningful and an original approach towards answering several problems in basic plant science, as well as in applied areas of environment for the better yield of crops, and for the betterment of nation due to very high cost of chemical nutrients in comparison to biofertilizers.

**AIMS AND OBJECTS OF THE STUDY**

The proposed investigation presented in this thesis is coined to study the effect of biofertilizers (blue green algae) on plants (paddy and tomato) with special reference to nutrient elements using crop plants. The experiments being aimed to investigate:

(i) The effects of graded supply of blue green algae as biofertilizers on growth and composition of plants.

(ii) Whether plants are in different stages of growth, different plant parts show the same response to supply of blue green algae as biofertilizer levels.

(iii) Whether biofertilizers have the same effect on different crop plants.

(iv) The optimum supply level of the blue green algae as biofertilizers for improved plant yields.