EVALUATION OF BLUE GREEN ALGAL STRAINS IN RELATION TO CARBON AND NITROGEN CONTRIBUTION

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EVALUATION OF BLUE GREEN ALGAL STRAINS IN RELATION TO CARBON AND NITROGEN CONTRIBUTION

By

K. SWARNALAKSHMI

A thesis submitted to the faculty of Post-Graduate School,
Indian Agricultural Research Institute, New Delhi
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This is to certify that the thesis entitled "**Evaluation of Blue Green Algal Strains in Relation to Carbon and Nitrogen Contribution**" submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **Microbiology** embodies the results of *bonafide* research work carried out by **Ms. K. Swarnalakshmi**, under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information that has been availed of in this connection has been duly acknowledged by her.

P. K. Singh

(**P. K. Singh**)
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Place : New Delhi
Date : 10th Oct., 2003

Dedicated to my parents

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To my teachers
And only the matter shall guide me, and only the master shall teach
And no one shall work for money, and no one shall work for fame,
But each for the joy of working, and each, in his separate star,
Shall draw the thing as he see for the God of things as they are.

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Place: New Delhi
Date : 10th Oct 2003

K. Swarnalakshmi
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Introduction

1. INTRODUCTION

The two inevitable dangers of resource depletion and environmental degradation are the consequences of overexploitation of nature. Rapid pace of industrialization has accelerated the process of environmental pollution. Present day agriculture, which is increasingly becoming chemical based and input intensive, has developed into an industry, contributing appreciably to the resource depletion and contamination of ground water. The impeding energy crisis due to fast depleting mineral oil reserves has on one side necessitated the search for renewable energy resources to ease the pressure on fuel energy. On the other hand, the increasing concern about the long-term productivity of agro ecosystems has equally emphasized the need to develop a management strategy that maintains and protects the soil resources. The latter issue is directly related to the maintenance of the level of soil organic matter and nitrogen status, which are the critical components of soil productivity.

Soil organic carbon and nitrogen are important indicators of soil quality and essential for sustaining agricultural production (Doran *et al.*, 1994). These parameters can change over time due to modification in soil and crop management practices such as cultivation, crop rotation, residue management or fertilization which, in turn, can influence the long-term sustainability of ecosystem (Campbell *et al.*, 1991).

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Carbon and nitrogen cycling seems to be of particular relevance for gaining and making use of practical knowledge in intensive rice soil management. Phototrophic primary production in the floodwater of wetland rice systems can account for 0.2-1.0 g C m^{-2} d^{-1} (Roger, 1996). Furthermore, anoxic bulk soils of wetland harbor vast amount of potential inorganic electron donors for chemotrophic biomass production.

The level of organic matter in soil is considered to be a good function of net input of organic residues by the cropping system and the other microbial processes (Gregorich *et al.*, 1994). Soil organic matter has a significant role to play in the sustainability of farming systems (Swift and Woomer, 1993) and also this parameter is an important indicator of soil quality and productivity (Larson and Pierce, 1994). Much information is available on the build up and turnover of soil organic matter and its various fractions as influenced by organic residues, fertilization and cropping pattern under temperate conditions (Liang and Mackenzie, 1992; Angers *et al.*, 1993), but very few studies have been conducted under tropical conditions where the turnover rate of organic matter is comparatively rapid.

Algae, a heterogeneous assemblage of plants, includes both prokaryotic and eukaryotic forms have been found to synthesize 0.8 x 10^{11} tonnes of organic matter, constituting about 40% of the total organic matter synthesised annually on this planet (Goyal, 2002). De

and Sulaiman (1950) claimed a build up of organic matter due to algal inoculation in paddy soil, which was supported later with experimental data of other researchers (Sankaram, 1971; Das *et al.,* 1991). Well-developed colonies of blue green algae (BGA) with wide variations in the levels of organic carbon or biomass have been reported in paddy (Osmanova, 1979). According to conservative estimates, even an increase of 0.1% in soil organic carbon can sequester 15 tonnes ha^{-1} of atmospheric carbon (Velayutham *et al.,* 2000). The fact that wetland rice systems can produce 2-4 tonnes ha^{-1} of grain yield without any fertilizer input (De Datta, 1987) highlights at the same time the significance of microbial biomass in its dual role as a labile sink and source of nutrients in submerged rice systems (Inubushi *et al.,* 1997a, b). These microorganisms add, conserve and mobilize crop nutrients in the soil.

On the other hand, biological nitrogen fixation, an attribute exhibited by some of the microorganisms, holds promise to meet at least a part of the nitrogen required for agricultural production. BGA (cyanobacteria), the photoautotrophic prokaryotes that are frequently considered as predominant diazotrophs in wetland rice systems, play a positive role in the sustenance of the nitrogen status of rice fields in our country (De, 1939). Venkataraman (1966) initiated the work on the algalization of the Indian rice fields. The All India Co-ordinated projects on the utilization of blue green algae in rice fields throughout the country revealed that the supplementation of chemical fertilizer

with blue green algae could conserve up to 30% of commercial chemical fertilizers (Venkataraman, 1972, 1981). In rice ecosystems, these are known to fix an average of 27 kg N ha^{-1} in N-free plots (Roger and Ladha, 1992; Carreres *et al.*, 1996). The rate is reduced to 8 kg N ha^{-1} when urea is broadcast (Roger and Ladha, 1992). It is generally believed that the nitrogen fixed by these organisms is made available to the rice plants through exudation or autolysis and microbial decomposition (Roger and Kulasooriya 1986; Querijero-Palaepac *et al.*, 1990).

In addition to contributing biologically fixed nitrogen and adding organic matter to soil, these organisms are also known to excrete growth-promoting substances, solubilize insoluble phosphates, improve the fertilizer use efficiency of crop plants and amend the physical and chemical properties of soil. These have been shown to have ameliorating effects on saline and saline alkali soils, increasing soil aggregate size thereby, correcting soil compaction, reducing the oxidizable matter content of the soil and narrowing down the C: N ratio (Goyal, 1993). This induced efficient management of the crop nutrients leads to long-term sustainability in crop production.

Sustenance of fertility status of soils is largely microbe mediated function of soil. Linking these processes with soil characteristics and biological attributes over a range of redox conditions is likely to provide considerable insights that are required for improvement in N management of rice soils. Information on carbon and nitrogen

balance under rice cropping system inoculated with blue green algal biofertilizer is lacking. Therefore, the role of blue green algal biofertilizer strains needs to be analyzed and examined on soil microbial profile and biomass, carbon-nitrogen content, physico-chemical and biological activities of the soil and their influence on growth and productivity of rice. Systematic studies are required to understand the pattern of bio geochemical functions of these biofertilizer strains in terms of carbon and nitrogen contribution, so that properly managed rice systems can ultimately become sustainable in the present day agricultural scenario.

Hence, the present investigation was undertaken with the following objectives:

1. To examine the influence of blue green algal inoculation on the soil microbial flora.
2. To evaluate the effect of blue green algal inoculation on certain important soil properties.
3. To analyze the impact of blue green algal inoculation on carbon and nitrogen contents in soil and rice crop.
4. To understand the relevance of blue green algal inoculation on growth and yield attributes of rice crop.

Review of Literature

2. REVIEW OF LITERATURE

Rice is one of the most important crops of South East Asia and covers more than 42 m ha, consuming more than 60 lakh tonnes of nitrogen in India (Nayak *et al.*, 2001). The successful production of rice depends upon efficient and economic supply of nitrogen, an element required in the largest quantity in comparison with other essential ones. The nitrogenous fertilizer use efficiency is low because of its loss from soils through various chemical and biochemical processes. Besides, increasing application of nitrogenous fertilizer is neither environmental friendly (Conway and Pretty, 1988) nor economically viable (Cassman and Pingali, 1994). It has therefore, become necessary to look for alternative renewable sources to meet at least a part of the nitrogen demand of rice crop (Swaminathan, 1982). In this context, biological nitrogen fixation is especially important in rice field where blue green algae (BGA)/cyanobacteria are recognized as significant contributors to the overall nutrient balance (Roger *et al.*, 1993).

Blue green algae constitute the largest, most diverse and widely distributed group of prokaryotes that perform oxygenic photosynthesis and many are capable of fixing atmospheric nitrogen, which helps to maintain and improve productivity of rice fields (Roger *et al.*, 1993). These are important components of soil especially in flooded rice fields, which are known to have a diverse

flora of morphologically distinct forms (Watanabe, 1959; Rogers and Kulasooriya, 1980). These organisms are able to withstand extremes of temperature and drought and show remarkable variation in growth, nitrogen fixation and stress compatibility (Goyal, 1997). In addition, these organisms have also been recognized as important agents in the stabilization of soil surfaces (Bailey *et al.,* 1973) primarily through the production of extracellular polysaccharides, which are prominent agents of aggregate formation, and stabilization (Molope *et al.,* 1985; Burns and Devies, 1986). The importance of polysaccharide in soil aggregation may be the direct result of binding soil particles into micro aggregates (Cheshire *et al.,* 1983, 1984), although Tisdall and Oades (1982) considered polysaccharides as only transient adhesives.

The need for algal inoculation arose from an earlier belief that N_2-fixing BGA strains were not widely present in rice fields. Occurrence of BGA was reported in only 5% of 911 soil samples (Watanabe and Yamamoto, 1971), 33% of 2213 samples (Venkataraman, 1975), and 71% of Japanese soils (Okuda and Yamaguchi, 1952). Algalization has been recognized as an important input in rice cultivation as it forms perpetually renewable source of nutrients and improves soil health (Singh, 1961; Venkataraman, 1981). Inoculation of rice fields with BGA was initiated in Japan in the early 1950s by Watanabe *et al.* (1951) and it was further developed in India, Burma, Egypt and China.

Considering the fact that 87% of the rice area in our country accounts for holdings of 1-4 ha and 13% of the holdings even less than 1 ha, an inexpensive rural oriented algal biofertilizer technology for rice was developed (Venkataraman, 1981). The method was simple, inexpensive, and easily adopted by farmers and the inoculum consisted of several species of the genera *Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena* and *Plectonema*. It was propagated by farmers in shallow trays or tanks with 5-15 cm water, about 4 kg soil m^{-2}, 100 g triple superphosphate m^{-2}, and insecticide. In one to three weeks, a thick mat developed on the soil or water surface. The trays were dried, and algal flakes scraped off and stored in bags for use in the fields (10 kg ha^{-1}). However, the production capacity of BGA flakes in India was only 0.01% of the total inoculum requirement for the country (Subba Rao, 1982). A similar method was used in Egypt, but only the floating algal flakes, relatively free of soil, were collected and dried. About 250 g ha^{-1} of dried algal flake was inoculated a week after transplanting rice (Watanabe, 1986). The blue green algal biofertilizer production has been further improved to get quality inoculum (Pabbi *et al.*, 2000). This can be easily produced in a polyhouse (Plate 1a) under semicontrolled conditions in cemented tanks (Plate 1b). The inoculum has been drastically reduced from 10 kg ha^{-1} to 1.5 kg ha^{-1} with the development of new material (Plate 1c).

Plate 1a. Polyhouse for indoor production of BGA biofertilizer

Plate 1b. Inside view of polyhouse

Plate 1c. BGA Biofertilizer packets

a

b

c


ALGAL
BIOFERTILIZER
FOR RICE
N N
ALGAL
BIOFERTILIZER
FOR RICE
N N
ALGAL
BIOFERTILIZER
FOR RICE
N N

2.1 Organic matter and C content

For several decades there has been a concern about soil organic matter and sustainability of the soil as a resource in intensive farming systems. There is always a consensus that soil organic matter has a significant role to play in the sustainability of farming systems (Swift and Woomer, 1993) and also this parameter is an important indicator of soil quality and productivity (Larson and Pierce, 1994). Gregorich *et al.* (1994) indicated that soil organic matter should be viewed as a set of fractions rather than a single entity. These fractions are descriptive of the quality of soil organic matter and the important fractions of organic matter are the light fraction, macro organic matter (particulate carbon), microbial biomass carbon, mineralizable carbon, carbohydrates and enzymes.

Soil microbial biomass within specific limits can serve as an indicator of organic matter change (Carter *et al.*, 1999). Other labile soil organic matter fractions such as macro organic matter and light fraction are highly responsive to change in carbon inputs to the soil and will provide measurable change prior to any change in total organic matter (Gregorich and Janzen, 1996). Campbell *et al.* (1997) and Bolinder *et al.* (1999) illustrated the sensitivity of several soil organic matter fractions to changes in carbon inputs. However, validity of this method is restricted under conditions where climate impedes adequate carbon inputs (Mele and Carter, 1993) or suppresses the rate of decomposition (Janzen *et al.*, 1998).

Within any one soil type, increasing carbon inputs via agricultural management is the key to increasing soil organic matter (SOM)) quantity (Jenkinson, 1990). The level of SOM may increase linearly with increase in input levels, although the slope of line depends on climate, soil type and soil management (Parton *et al.*, 1996). Higher soil organic matter content has been found to have positive effects on yield and yield components in cereals (Gorlitz and Asmus, 1984; Schneider, 1984; Gorlitz, 1986) as well as on soil density, pore volume and maximum water holding capacity (Asmus *et al.*, 1987).

The mineralization of soil organic matter provides a continuous and limited supply of nitrogen to the crop and generally the added organic matter with the C:N ratio less than 20 releases mineral nitrogen. The organic manures used with C:N ratio between 7-19 are thus in a suitable range for mineralization (Tisdale and Nelson, 1975; Singh *et al.*, 1981). Rapid release of ammonium nitrogen was reported in soils rich in organic matter (Ponnamperuma, 1977).

Recently it has been established that more dynamic characteristics such as microbial biomass, soil enzyme activity and soil respiration respond more quickly to changes in crop management practices or environmental conditions than the characteristics such as total soil organic matter (Brookes, 1995). Much information is available in the build up and turnover of soil organic matter (Liang and Mackenzie, 1992; Angers *et al.*, 1993). But very few studies have been conducted under tropical conditions, where the turnover rate of soil

organic matter is comparatively rapid (Ayanaba and Jenkinson, 1990; Goyal *et al.*, 1993).

2.2 Studies in BGA

Little attention has been given to the general features of physiology of blue green algae with the specific attention given to nitrogen fixation and some aspects of photosynthesis (Fogg, 1947; Brown and Webster, 1953). A number of studies have used blue green algae as reliable tools for studies involving growth and other related parameters (Kratz and Myers, 1955). A wide variation was observed in twenty-eight non-heterocystous filamentous blue green algal strains with respect to dry weight, generation time and tolerance to biocides (Tiwari *et al.*, 2000, 2001).

The absorption of light energy by these organisms is based upon the occurrence of one or two forms of chlorophyll with chlorophyll *a* as pivotal pigment together with carotenoids and phycobilins as accessory pigments. Accessory pigments confer extended ability to harvest light for photosynthesis and, in some cases, protection from UV and other light-induced cell damage. Chlorophylls are associated with membranous thylakoids similar to those of plants and other algae. The association of carotenoids with chlorophyll prevents the formation of highly reactive singlet oxygen radicals that would otherwise cause irreparable damage to lipids, proteins and other molecules (Bartley and Scolnick, 1995). These organisms are also known to possess water-soluble phycobilin

pigments that transfer captured energy to chlorophyll *a*. Three types of phycobiliproteins are produced *viz.*, phycocyanin (Blue), allophycocyanin (Blue grey) and phycoerythrin (Red). However, not all taxa that contain phycobiliproteins produce all the three types (Graham and Wilcox, 2000).

2.3 Nitrogen fixation by Blue Green Algae

Nitrogen fixation is carried out in specialized cells known as heterocysts, which have thick walls and hence, physically prevent the entry of oxygen and provide necessary anaerobic conditions for the activity of nitrogenase enzyme. These cells have a high rate of respiration that scavenges the diffused oxygen, and they lack photosystem II, due to which there is no splitting of water and evolution of oxygen during photosynthesis (Thomas, 1970). Till the findings of Wyatt and Silvey (1969), only heterocystous forms were considered to be capable of fixing nitrogen. Since then, many of the non-heterocystous forms have also been reported to fix nitrogen under anaerobic or microaerophilic conditions except *Gloeocapsa*, which is an aerobic nitrogen fixer (Stewart *et al.*, 1979). Stewart and Lex (1970) suggested that all the vegetative cells of trichomes of diazotrophic blue green algae contain nitrogenase but enzyme gets inactivated in the presence of oxygen.

Once nitrogen is fixed, many organisms are known to incorporate ammonia into amino acids by the enzyme glutamate dehydrogenase and this enzyme has been found to be absent or

present in low amounts in blue green algae (Hoare *et al.*, 1967; Pearce *et al.*, 1969). Under the conditions of ammonia limitation, most prokaryotes use a pathway consisting of glutamine synthetase and glutamate synthase to assimilate ammonia (Miflin and Lea, 1976). In blue green algae, this pathway has shown to be the major ammonia assimilatory route under nitrogen fixing conditions (Thomas *et al.*, 1975; Wolk *et al.*, 1976).

2.4 Factors affecting BGA growth

Blue green algae have to function under the conditions where they are always exposed to the vagaries of the environment which can be natural or man made. Most of these are reported from tropical soils although BGA are also found to occur frequently in temperate soils (Reynaud and Metting, 1988).

2.4.1 Effect of N

Numerous reports are available on N fertilizer inhibition of BGA growth (Singh, 1975, 1985; Roger and Kulasooriya, 1980). Fertilizer nitrogen had a differential effect on growth and nitrogen fixation in blue green algae (Goyal and Marwaha, 1985; Goyal, 1989). Field studies conducted at CRRI, Cuttack indicated that *Aulosira* produced biomass amounting to 176 and 212 kg dry weight ha^{-1} at 30 and 50 days after inoculation at zero level of N whereas at 120 kg N ha^{-1} (urea), the biomass was reduced to one fourth (Singh and Bisoyi, 1989). Trials conducted by Singh and Singh (1986) indicated that ammonium and urea N above 30 kg N

ha^{-1} retarded the increment in biomass and N yield of BGA in unplanted rice fields. Stewart (1964) and Venkataraman (1979) did not observe any reduction in nitrogen fixation in the presence of 50 ppm N.

A readily available source of exogenous ammonium salts suppressed nitrogen fixation and the extent of inhibition by other nitrogenous compounds depends on the ease with which they release ammonia (Allen, 1956; Fogg, 1942; Wilson and Burris, 1947). Exposure of blue green algal strains (from mid log phase) to 1 mM NH_4Cl resulted in a sharp repression of nitrogenase activity to an extent of 70 – 85 per cent within 24 hours. Nitrogenase activity, however, rapidly revived once the repressor (NH_4Cl) was removed from the medium (Dhar and Pabbi, 1993). According to Stewart (1980), in blue green algae the repression of nitrogenase enzyme was not regulated by glutamine synthetase, instead presence of ammonia increased ADP/ATP ratio and carbamoyl phosphatase activity, which in turn cumulatively and independently inhibited the nitrogenase activity. Removal of ammonium nitrogen stress reduced the ADP/ATP ratio to less than 0.05 and brought down the carbamoyl phosphatase activity, which derepresses the nitrogenase enzyme and revival of nitrogenase enzyme took place within 24 hr of removal of the stress (Chandrasekaran and Venkataraman, 1985). Ammonium nitrogen is the putative inhibitor of nitrogen fixation and proved toxic for algal growth at 75 ppm (Stewart, 1964; Goyal and Marwaha, 1985).

2.4.2 Temperature

Roger and Kulasooriya (1980) and Singh (1976) observed that the temperature of 34-39°C was favorable for growth of *Aulosira fertilissima* in rice fields. Deleterious effect of high temperature has also been reported (Venkataraman, 1964) and low temperature retardation of growth of blue green algae have also been shown by some specific studies (Roger and Reynaud, 1979).

2.4.3 Light

Being photoautotrophic, blue green algae show definite response to the quantity and quality of light, although quite a few members could exhibit growth and fix nitrogen even in the dark (Fay, 1965; Fay and Fogg, 1962; Kiyohara *et al.*, 1960; Venkataraman, 1961). Low light intensities are generally preferred by blue green algal strains; however, certain forms like *Cylindrospermum* (Traore *et al.*, 1978) and *Aulosira* (Singh, 1976) grew well under high light intensities. Half saturation of nitrogen fixation by *Tolypothrix tenuis* was obtained at intensity lower than 800 Lux (Ukai *et al.*, 1958).

2.4.4 Water and Desiccation

Most forms are known to survive periods of desiccation with little obvious morphological change. *Nostoc muscorum* and *Nodularia harveyana* have a high capacity to withstand desiccation. This ability has been attributed to various physiological and morphological parameters like plasmolysis and lack of cell vacuoles.

Formation of cysts in some genera and presence of mucilaginous sheath which may absorb water quickly and retain it for a longer period can also be important factors to resist desiccation (Roger and Reynaud, 1982). This explained dominance and mucilaginous colonies of *Nostoc* sps. and *Cylindrospermum* sps. (Traore *et al.*, 1978) in the rice fields with dry or moist but shaded soils.

The rewetting of dried *Nostoc commune* lead to recurrence of physiological processes like respiration, photosynthesis and nitrogen fixation (Scherer *et al.*, 1984). A non-colonial culture of *Nostoc commune* showed increased nitrogenase activity and size of intracellular ATP pool when rewetted, and the up shift in nitrogenase activity was preceded by a lag (Potts and Bowman, 1985). Rains (Singh, 1976) and typhoons (Roger and Kulasooriya, 1980) affected the growth of blue green algae by reducing availability of light and washing out the blue green algal biomass. Seasonal variation in parameters like temperature, solar radiation, and sunshine hours showed positive correlation and rainfall showed negative correlation with growth and N yield of blue green algae (Bisoyi and Singh, 1988).

2.4.5 pH

The pH of the soil/water influence growth of blue green algae. These organisms in general, are reported to exhibit ideal growth in the pH range of 6.5-8.5 (Singh, 1974). The nitrogen fixing alga *Anabaena iyengarii* isolated from soil with pH 4.8 did not grow at higher pH. But another strain isolated from soil with pH 5.5 responded favorably to

increase in pH (Sardeshpande and Goyal, 1981). *Cylindrospermum sphaerica* failed to grow below pH 6.5, but with increasing pH, growth and nitrogen fixation have been found to increase upto a pH level of 8.0 (Venkataraman, 1961).

In another study on rice field soil with pH 10, a strain of *Anabaena variabilis* was found to have growth optima at pH 9.0. The beneficial effect of liming was reported to be closely related to pH rather than to the availability of calcium (Roger and Reynaud, 1979). Liming increased the pH from 6.5 to 7.5, which enhanced the availability of several nutrients in flooded soil that benefited growth, nitrogen fixation and establishment of inoculated blue green algae (Amma *et al.,* 1966; Saha and Mandal, 1980; Singh, 1961; Subramanyan, 1972; Venkataraman, 1972). However, trials conducted at CRRI, Cuttack showed that the liming up to 1500 kg ha^{-1} had no appreciable effect on growth and N yield of blue green algae (Singh and Bisoyi, 1989). BGA also caused diurnal fluctuations in soil and water pH (Katyal and Carter, 1989; Mikkelsen *et al.,* 1978) through utilization of CO_2 for photosynthesis during daytime and release of CO_2 by respiration during nighttime.

2.4.6 Effect of potassium and molybdenum

Potassium fertilizers applied at high doses of 100 kg ha^{-1} did not show any stimulatory or inhibitory effect on growth and N yield of blue green algae (Singh and Bisoyi, 1989). Wilson and Alexander (1979) also did not get any correlation of potassium content of soil with ARA activity. There was no appreciable effect of

molybdenum on growth and nitrogen fixation by blue green algae under field studies conducted at CRRI, Cuttack probably because, the Indian soils are rarely deficient in molybdenum and the requirement of this element is met in the rice fields (Singh and Bisoyi, 1989). However, Fogg (1956) has explained the possible implications of molybdenum in nitrogen fixation and nitrate reduction.

2.4.7 Pesticides

A wide tolerance of blue green algae to a variety of pesticides has been reported (Venkataraman and Rajalakshmi, 1971; Ahmad and Venkataraman, 1973; Singh, 1973; Da Silva *et al.*, 1975) and depending upon the nature and concentration of the chemicals, their effect may be stimulatory (Ahmad and Venkataraman, 1973) or inhibitory (Venkataraman and Rajalakshmi, 1971). In a study with four commonly used weedicides in rice cultivation, the response in terms of growth and nitrogen fixation varied with the organisms and type and concentration of weedicide used. Out of Butachlor (Machate), Benthiocarb (Saturn), Pandimethalin (Stomp) and Oxadiazon (Romstar), only Pandimethalin affected growth and nitrogen fixation at higher concentration (Goyal, 1989). The toxicity of insecticide decreased with the incubation period, which suggests detoxification by blue green algae (Das and Singh, 1977). Gamaxene and BHC were found to be algicidal to various bloom

forming blue green algae at 10 ppm concentration (Das and Singh 1978, 1979).

2.4.8 Grazing

Several reports have attributed to the disappearance of inoculated blue green algae in the fields due to grazing (Singh, 1981b, 1985). Grabbour *et al.* (1981) observed that protozoa and nematodes retarded the growth of BGA. Roger and Reynaud (1982) observed that *Lymnaea*, a snail was major predator of BGA in a rice field of Philippines. Mucilage forming colonies of blue green algae were less susceptible to grazing than non-mucilage forming ones (Singh, 1981a; Grant *et al.*, 1986). *Cypris* sp. and *Osteracod* have been documented as BGA predators (Wilson *et al.*, 1980; Osa-Afiana and Alexander, 1981; Grant and Alexander, 1981).

2.5 N contribution by blue green algae and soil fertility

Soil nitrogen pool is believed to be maintained through biological nitrogen fixation (Kundu and Ladha, 1995; Roger and Ladha, 1992) and fertilizer nitrogen. Jeffries *et al.* (1992) suggested that soil algae play a major role in nutrient cycling in desert and semi desert ecosystem, especially in cycling of nitrogen. Among indigenous nitrogen fixers in rice fields, blue green algae are the main contributors to nitrogen fixation (Roger and Ladha, 1992). Nitrogen is brought into organic farming systems through the inclusion of nitrogen fixing crop in rotation or use of biofertilizers/

blue green algae in rice crop. As a result, nitrogen balance studies under such systems are usually positive (Nguyen *et al.*, 1995). A blue green algal bloom usually corresponds to less than 10 Kg N ha^{-1}, a dense bloom may contain 10-20 kg N ha^{-1} (Roger, 1991).

Blue green algal biofertilizer is recommended only as a supplement to nitrogenous fertilizers and the supplementation effect may remain perceptible even in the presence of high levels of fertilizer nitrogen (Venkataraman and Goyal, 1969). Pronounced additive effect of algal application at lower levels of fertilizer nitrogen becomes important in extensive agriculture (Goyal, 1982, 1989) which envisages use of less fertilizer nitrogen on larger areas for reducing loss of fertilizer nitrogen and ensuring maximum utilization of the natural process.

Importance of fixation of nitrogen and sustenance of nitrogen fertility of soil has been reported by Singh, 1961; Singh and Bisoyi, 1989; Santra, 1993. Lot of information has been generated in tropics regarding improvement in the fertility status of rice soils to sustain rice yields by utilizing diazotrophic blue green algae as the biological input (De, 1939; De and Sulaiman, 1950; Venkataraman, 1972; Singh and Bisoyi, 1989). These organisms gave a considerable build up of nitrogen fertility in rice soil (Roger and Kulasooriya, 1980; Saha and Mandal, 1980; Roger and Reynaud, 1982). Investigations have also been undertaken with regard to the possibility of using nitrogen-fixing cyanobacteria in non-flooded temperate agricultural soils (Reynaud and Metting, 1988).

Multilocational trials conducted under varying agroclimatic conditions using different rice varieties indicated that algal inoculation can result in an addition of 30 kg N ha^{-1}. This however, depends upon agroecological condition, which would regulate the activity and establishment of introduced algae (Venkataraman, 1979; Venkataraman and Goyal, 1969), though Roger and Kulasooriya, (1980) and Singh and Singh, (1987) recorded 30 kg N ha^{-1} $year^{-1}$ as a satisfactory value when environmental factors are favourable. Experiments conducted at CRRI, Cuttack indicated that inoculation in soil with *Aulosira* sp. at the rate of 60 kg ha^{-1} (fresh weight) registered significant changes of soil nitrogen content. BGA incorporated to soil increased 13-14% of N content under field conditions and BGA amended soil released 50% of ammonium N at 50 days of flooding (Singh *et al.*, 1981). The rate of N released by BGA was 12 and 35% after 7 and 35 days of flooding (Saha *et al.*, 1982). Ghosh and Saha (1997) also reported that the inoculation of soil with soil based mixed culture of four diazotrophic cyanobacteria namely *Aulosira fertilissima, Nostoc muscorum, Nostoc commune* and *Anabaena* species significantly increased the release of inorganic nitrogen in soil. Nitrogen content of soil was higher in exposed light incubated soil than unexposed soil due to N gain by blue green algae (Singh and Singh, 1987).

Inoculation with *Nostoc muscorum* in a green house experiment had a pronounced effect on soil chemical and biological properties with total nitrogen increasing by 111-120% (Rogers and

Burns, 1994). Chopra and Dube (1971) reported that the pots inoculated with *Tolypothrix tenuis* showed considerable increase in total and organic nitrogen. Release of nitrogen from rapid decomposition of fresh or dry mass incorporated into the soil has been reported (Saha *et al.*, 1982; Tirol *et al.*, 1982; Miam and Stewart, 1985).

2.6 Estimation of nitrogen fixing potential

Tropical conditions ensure increased incidence of blue green algae in rice field soils because high humidity, temperature and shade provided by crop canopy favour the luxuriant growth of these organisms (Roger and Reynaud, 1979). The evidence for nitrogen fixation has come from long-term fertility trials (Watanabe *et al.*, 1981), N balance studies (Ventura and Watanabe, 1983) and experiments involving acetylene reduction assay (Yoshida and Ancajes, 1971). Accurate reproducible measurement of N fixing potential of BGA as an estimate of N increment are extremely important in field studies especially in rice cultivation (Watanabe *et al.*, 1977; Roger and Ladha, 1992; Roger, 1996). Significance of phototrophic N fixation in tropical rice fields was recognized using acetylene reduction assay and total N measurements (Rice and Paul, 1971).

ARA is a sensitive tool to detect nitrogenase activity but its accuracy for quantification studies has been much debated (Lee and Watanabe, 1977; Roger, 1996). Nitrogen fixation by blue

green algae has been predominantly estimated and data published before 1980 varied from a few to 80 kg N ha^{-1} $crop^{-1}$ (Mean 27 kg) (Roger and Kulasooriya, 1980). About 200 crop cycle measurements in experimental plots at IRRI showed the activities as 0-1200 μmol C_2H_4 m^{-2} h^{-1} for daily values and 20-500 μmol C_2H_4 m^{-2} h^{-1} for average ARA during a crop cycle. Extrapolated values (assuming $C_2H_2/N_2=4$) ranged from 0.2 to 50 kg N ha^{-1} $crop^{-1}$ and averaged 20 kg in no-N control plots, 8 kg in plots with broadcast urea and 18 kg in plots where N was deep placed. ARA was negligible in 75% of 60 plots where urea was broadcast (Roger *et al.*, 1988). Watanabe and Cholitkul (1977) have also described various methods for field estimation of biologically fixed nitrogen and reported an addition of 18-45 kg N ha^{-1} due to the activity of these diazotrophic organisms, using ARA technique (Watanabe and Cholitkul, 1977). They observed that the improvement and modifications in the methodology for ARA were needed for accurate measurements. Experiments carried out over a period of three consecutive years revealed that the estimates of nitrogen fixation were comparable with the results of other workers using a simple and modified technique which can provide rapid and reliable measurements of nitrogen fixation under field conditions (Prasanna *et al.*, 2003).

Availability of nitrogen fixed by blue green algae to the rice plant has been shown with the help of ^{15}N studies (Reynault *et al.*, 1975; Inubushi and Watanabe, 1986). Using ^{15}N, Mayland and

McIntosh (1966) and Stewart (1967) have shown the possible contribution of blue green algal nitrogen fixation. Miam and Stewart (1985) observed that about 50% of total N fixed by BGA is released to the surroundings.

Recently, contribution of N_2 fixing blue green algae to rice production and availability of nitrogen using ^{15}N labelled material, in microplot experiment to obtain more direct information on the dynamics of utilization of N by rice plants has been studied (Fernandez-Valiente *et al.*, 2000). In this study, the recovery of blue green algal nitrogen was compared with the recovery of same amount of labelled ammonium sulphate under field conditions. The availability of nitrogen to rice plant was similar to that of chemical fertilizer even at the tillering stage indicating a fast mineralization of organic nitrogen in the soil followed by a rapid and fast transfer of fixed nitrogen to rice crops. The amount of blue green algal nitrogen recovered in plants was however lower in other study (Tirol *et al.*, 1982).

2.7 Influence of blue green algal inoculation on N uptake

In intensified rice systems N use and N uptake efficiency decreases as application of N fertilizer increases (Carreres *et al.*, 1996). The role of blue green algae in nitrogen economy of rice fields and the yield of rice has been well demonstrated and widely documented (Venkataraman, 1981; Santra, 1991). Nitrogen fixed by these organisms may become available to rice plants only after

their release into the surrounding either as extracellular products and/or on mineralization of the intracellular contents. Direct evidence of the transfer of blue green algal N to rice plants are however, scarce (Roger, 1996). Nitrogen fixation by blue green algae *vis a vis* its release in the soil water system may be more useful for crop production during the vegetative growth stage of rice plants than at later stages (Ghosh and Saha, 1993; Roger *et al.*, 1993).

Recovery of BGA fixed N by rice varied from 13-50% depending upon the nature of inoculum, method of application and the absence of soil fauna (Tirol *et al.*, 1982). 52% of nitrogen added was recovered in the grain and straw of the first rice crop using suspension of blue green alga, *Aulosira* buried 5-7 cm deep in green house pot culture experiments. Addition of ammonium chloride equivalent to 100 kg N ha^{-1} did not affect the recovery of algal nitrogen and surface placement of alga reduced the recovery by first crop to 37% of added nitrogen (Wilson *et al.*, 1980). The results from the studies undertaken by other workers also indicated the transfer of fixed nitrogen from blue green algae to higher plants and demonstrated the potential for efficient transfer of nitrogen from algal cells to rice plants (Jones and Wilson, 1978; Mayland and McIntosh, 1966; Stewart, 1967). In a four crop experiments comparing the role of *Azolla*, blue green algae and urea, Singh and Singh (1987) found positive N balance ranging from 13-163 mg N $crop^{-1}$ pot^{-1}. Balances were highest (133-163 mg N $crop^{-1}$ pot^{-1}) in pots that

received 60 kg organic nitrogen (BGA and *Azolla*) ha^{-1} and lowest 13-29 mg in pots that received 30-60 kg N ha^{-1} as urea. Balance in the control was 51 mg N $crop^{-1}$ pot^{-1}.

2.8 Influence of BGA on crop yield

Effect of algalization on grain yield under field conditions has been reported from China (Ley, 1959), Egypt (El-Nawawy and Hamdi, 1975), Japan (Watanabe, 1965) and India (Jha *et al.*, 1965; Venkataraman, 1972; 1979; 1981; Singh, 1978; 1985; 1988; Chopra *et al.*, 1985; Kaushik, 1985). The results as reviewed by Roger and Kulasooriya (1980) revealed an average increase of 14% in rice yield over control which was equivalent to the application of 25-30 kg N ha^{-1} as biofertilizer (Venkataraman, 1981). The potential for practical use of blue green algae as biofertilizer in rice farming was extensively discussed by Roger and Watanabe (1986). However, their inoculation failed to increase rice yields consistently (Watanabe, 1986; Roger *et al.*, 1993).

Field experiments at Cuttack during 1978-79 indicated that mixed inoculation of *Aulosira, Aphonothece* and *Gloeotrichia* increased grain yield by 10, 17, 32 and 35% during first, second, third and fourth crop (Singh, 1978). Singh and Singh (1987) also reported an increase of 15-23% grain yield by BGA inoculation. Under pot culture conditions, unialgal and composite cultures of blue green algae were able to increase the rice yield considerably as compared to control uninoculated conditions (Dhaliwal *et al.*, 1995).

Increased rice yield due to algal inoculation have been reported under control pot culture and field experiments (Relwani, 1963; Sundara Rao *et al.*, 1963; Singh, 1961; Venkataraman and Goyal, 1968). However, the amount of nitrogen fixed by blue green algae and its effect on growth and yield of rice crops varies with different doses of nitrogenous fertilizer (Venkataraman, 1972). Pandey *et al.* (1993) reported that the combination of 10 kg ha^{-1} BGA biofertilizer with 90 kg nitrogen as urea ha^{-1} resulted in better growth and maximum yield of the rice crop.

Arora *et al.* (1989) reported that blue green algal application to rice grown in pots could save 50% of the recommended dose of chemical nitrogen being applied to rice. Inoculation of soil based mixture of four heterocystous species led to an insignificant increase in grain (8%) and straw (11%) yield which was however accompanied by significant increase in nitrogen uptake by the grain (30%) and an increase in total uptake of 15.3 kg N ha^{-1} (Ghosh and Saha, 1997).

Ahmed (2001) reported that the deployment of BGA strains together with chemical nitrogen fertilizers increased grain yield of rice, in the fields of Nagaon-Sub division of Assam. It has also been shown that the combined application of blue green algae and N fertilizer is more effective in increasing the number of tillers and crop yield than the application of blue green algae alone in rice fields (Roychoudhary *et al.*, 1983; Patel *et al.*, 1984; Aiyer *et al.*, 1972). Sometimes the effect of BGA inocula on the yield of crops in

the presence of N fertilizer has been ascribed to production of growth promoting substances which can accelerate root growth (Brown *et al.*, 1956; Kopteva, 1970; Tupik, 1973; Venkataraman and Neelakantan, 1967). This in turn enables the crop plant to take up more nitrogen from the soil (Sundara Rao *et al.*, 1963). N fertilization along with the algalization stimulated plant growth and produced more photosynthetic area, which probably helped in increasing the crop yield (Watanabe *et al.*, 1951; Subrahmanyan and Manna, 1966; Jalapathi *et al.*, 1977; Yadav *et al.*, 1988).

2.9 Role of BGA on organic matter and C status

A build up of organic matter due to algal inoculation in rice soil has been reported (De and Sulaiman, 1950; Das *et al.*, 1991). Fuller and Rogers (1952) estimated an annual increment of 6 tons organic matter per million pounds of soil in Arizona through BGA inoculation. BGA inoculation increased soil organic carbon and Singh (1961) reported 68.7% increase of organic matter in Usar soils. Using ^{15}N, Nekrasova and Aleksandrova (1982) confirmed that algal biomass contributed significantly to humus formation in soils despite the absence of typical lignin in them. All these results and others compiled by Roger and Kulasooriya (1980) and Roger *et al.* (1987) indicated that under favourable conditions a good algal bloom in rice field yields, on average about 6-8 tonnes of fresh biomass. The persistence of such biomass in soil as organic matter however, depends on its decomposability. The biomasses of some

algae are decomposed quickly. While those of others last longer (Watanabe and Kiyohara, 1960). The differing susceptibility of algae to decomposition is related to the relative biodegradability of algal cell-wall compounds, like polyaromatic compounds (Gunnison and Alexander, 1975) and their physiological growth stages. As an example, the decomposability of *Anabaena* sp in soil is faster than other commonly inoculated BGA species in rice fields. Algal biomass rich in akinetes is also not easily decomposed when compared with algal vegetative cells (Mandal *et al.*, 1999).

Aiyer *et al.* (1972) could not detect any increase in organic carbon and attributed it to rapid loss of organic matter due to tropical climatic conditions. Further, in rice fields well-developed colonies of blue green algae with wide variations in the levels of organic carbon or biomass addition by BGA have been recorded (Osmanova, 1979; Rao and Burns, 1990; Das *et al.*, 1991). However, experiments at CRRI revealed 5-32% increase of soil organic carbon under field conditions (Singh *et al.*, 1981). Inoculation with *Nostoc muscorum* in a green house experiment was reported to enhance total C by 50-63% (Rogers and Burns, 1998).

2.10 Influence of blue green algae on soil properties

Roger and Kulasooriya (1980) described that the beneficial properties of rice-field soils may be enhanced by the growth of blue

green algae. The properties included improved water-holding capacity, release of vitamins or plant stimulating hormones, formation of extracellular polysaccharides leading to improved soil aggregation and solubilization of phosphates and significant improvement as measured in terms dehydrogenase, urease and phosphatase activities. Singh (1961) reported that the mucilaginous and fragile thalli of *Aphanothece* form a compact grey substratum firmly holding the soil particles together which also checks the soil erosion. Such improvement in soil aggregation due to algal inoculation may favour better seedling emergence of upland crops soon after rice harvest (Rogers and Burns, 1994).

BGA are known to excrete extracellularly a number of compounds like polysaccharides, peptides, lipids in soil, which possibly diffuse around soil particles and hold/glue them together in the form of micro aggregates (Marathe, 1972; Mehta and Vaidhya, 1978; Bertocchi *et al.,* 1990), which in turn grow and take the shape of macroaggregate and subsequently convert into larger soil aggregates. The importance of these compounds in soil aggregate formation or soil stabilization has been indicated by many workers (Schulten, 1985; Rogers *et al.,* 1991; Rogers and Burns, 1994). The quantity and quality of excreted compounds also vary depending upon the species of blue green algae, their physiological growth stages and also the associated environmental conditions (Mehta and Vaidya, 1978; Lama *et al.,* 1996).

Rao and Burns (1990) demonstrated that the inoculation of flooded brown earth silt loam with the consortia of blue green algae including *Nostoc muscorum* increased soil polysaccharide content by 69%, leading to a subsequent improvement in soil aggregate stability.

Some researchers considered polysaccharides as transient adhesives (Tisdall and Oads, 1982) and/or not directly involved in the formation of aggregates (Martens and Frankenburger, 1992) although the products of their microbial degradation like aliphatic and polyphenolic compounds are considered to be responsible for this function (Haynes *et al.*, 1991). Such polysaccharides are extracellular mucilages of blue green algae and can account for as much as 44% of dry weight (Moore and Tischer, 1964). Polysaccharides from different algal species may also vary with respect to protein, uronic acids and sugar composition and thus, in their stability in soils with respect to microbial and thermal degradation (Bertocchi *et al.*, 1990). Water stable aggregates, which are an integral part of aggregate formation have been shown to increase due to algal inoculation resulting in an improvement in the water holding capacity and aeration status of the soil (Singh, 1961; Marathe, 1972; Subhashini and Kaushik, 1981; Roychoudhury *et al.*, 1983; Tiwari *et al.*, 1991).

2.11 Oxygen concentration and associated changes

Blue green algae are aerobic photosynthetic organisms and release lot of oxygen during photosynthesis through photosystem II. As a result, when they grow in the rice fields they make the standing water highly oxygenated (Harrison and Aiyer, 1913). The concentration of oxygen in rice field flooded water normally varies around 4-6 $\mu g\ g^{-1}$ (Mandal, 1961; Saito and Watanabe, 1978; Krock *et al.*, 1988). Where there is profuse BGA growth, the oxygen concentration sometimes reaches 10-12 $\mu g\ g^{-1}$ (Mandal, 1961; Lakshmanan *et al.*, 1994) and the surface layer of soil absorbs enough oxygen through diffusion to become aerobic in nature and therefore, prevent the development of highly reduced conditions underneath. Under continuous flooded conditions, water logging creates intense reducing conditions with the redox potential value falling below -200 mV (Ponnamperuma, 1972). These conditions favour the formation and subsequent accumulation of high amount of harmful oxidizable organic matter, a large quantity of iron (Fe^{2+}) and also sulphur (S^{2-}) which sometimes reach toxicity levels for rice plants (Aiyer *et al.*, 1971 a, b).

Aiyer *et al.* (1972) reported significant reduction in oxidizable organic matter, total S^{2-} and Fe^{2+} contents of soil after four successive crops with BGA inoculation. A high oxygen tension in the standing water on soil arising from algal photosynthesis through pigment system II seems to facilitate the oxidation of these reduced components.

When applied or native NH_4-N in soils comes in to contact with the oxygenated surface layer, it is converted to NO_3-N, which on diffusion to the anaerobic layer is subjected to the process of denitrification and lost as gaseous nitrogen. Besides this, the rice rhizosphere itself is oxygenated due to transport of oxygen from the atmosphere to the soil through aerenchyma (John *et al.*, 1974), while the surrounding zone lacks oxygen, thus, making the environment highly favourable for nitrification-denitrification process (Garcia and Tiedje, 1982). Therefore, the extra oxygen received in the system from BGA makes the conditions more favourable for the denitrification loss of energetically costly N-input. On the other hand, it leads to the oxidation of Fe^{2-}, which sometimes occurs in the rice rhizosphere at a very high concentration and precipitate it as FeOOH forming a reddish coat on the roots, which may constitute up to 14% of the root dry weight (Chen *et al.*, 1980). Fe^{3+} and Fe^{2+} may have an inhibitory effect on denitrification in the rice rhizosphere, since these ions have shown some inhibitory effects on denitrification in laboratory studies (Komatsu *et al.*, 1978). Saha and Mandal (1979) observed a marked decrease in N-NH_4OAc in pH 3.0 - extractable iron along with a drastic fall in N-NH_4OAc, pH 7.0 extractable manganese in soils due to algal growth. Similar type of studies has also been conducted on the transformation of the Fe and Mn in submerged soils (Das *et al.*, 1991).

After completion of the growth cycle, the algal biomass may also undergo anaerobic changes resulting in the formation of

various organic acids causing changes in the oxidation-reduction status of the soils (Saha *et al.*, 1982).

2.12 Influence on microbial population

Soil biota are the important soil constituents and the measurements of their abundance, diversity or activity are considered potential indicators of soil quality (Gregorich *et al.*, 1997). Blue green algal abundance in soils has mostly been determined by direct observation, plating techniques and measurement of pigments (Roger and Kulasooriya, 1980). Tsujimura *et al*, (1998 a, b) investigated the distribution of soil algae in saline irrigation land using culture dilution method, which estimates density. However, the culture dilution method for soil algae estimation tends to lead to underestimation (Broady, 1979; Whitton, 2000). Another problem when estimating algal biomass by the culture method is that the sample may include organisms, which can grow in culture but not *in situ* conditions (Pandey, 1964). Some algae, which may inhabit water, form dormant cells such as zygospores in dry periods and then germinate when the habitat is resubmerged (Coleman, 1983). Other methods for quantifying soil algae have been attempted such as direct microscopic examination technique using fluorescent microscopy (Tchan, 1952), pigment extraction technique (Tchan, 1959; Johansen, 1993), implanted slide technique (Pipe and Cullimore, 1980), MPN method (Hunt *et al.*, 1979). However, each method has its own advantages and

limitations (Fogg *et al.*, 1973; Whitton, 2000) and till now no standard method for estimation of soil algae has been established (Hoffmann, 1989). Venkataraman (1975) reported that the number of microorganisms is affected in pot experiment using *Tolypothrix tenuis* as the inoculant.

Ibrahim *et al.* (1971) observed an increase in the total microbial population, especially the number of nitrifiers and *Azotobacter* and *Clostridium*. Rao and Burns (1990) observed an eight fold increase in bacterial number after 13 weeks of inoculation with the mixture of BGA. However, the increase was only 2.8 fold after 21 weeks. Similarly, Rogers and Burns (1994) recorded 500 fold, 16 fold and 48 fold increase in bacteria, fungi and actinomycetes population under the treatment inoculated with *Nostoc muscorum* over the non-inoculated one.

Bachinger (1996) investigated soil microbial parameters and reported that the treatment with high N content humus exhibited higher biological activity like protease and dehydrogenase activity as well as microbial biomass (Chloroform fumigation extraction). Microbial biomass is the main agent that supports the soil functions and associated processes involved with the storing and the cycling of nutrients and energy (Carter *et al.*, 1999).

2.13 Other effects

In addition to nitrogen fixation and carbon contribution, BGA inoculation increased the P availability in the soil and P amended

soil showed 21 ppm of P and highest P availability due to blue green algae was recorded at 21 days of incubation (Singh *et al.*, 1981; Saha *et al.*, 1982). Enhancement in rice seed germination, root - shoot growth, weight of rice grains and their protein content has also been observed (Shukla and Gupta, 1967; Venkataraman and Neelakantan, 1967; Singh and Trehan, 1973; Jacq and Roger, 1977) while others found similar stimulatory effect on wheat (Gupta *et al.*, 1967), tomato (Kaushik and Venkataraman, 1979; Rodgers *et al.*, 1979), peas (Gupta and Gupta, 1972), banana (Ganapathi *et al.*, 1994). Different opinions exist regarding the nature of these substances. Some have described them as hormones (Auxin like, Gibberellin like, Cytokinin like or Abscisic acid) (Singh and Trehan, 1973; Rodgers *et al.*, 1979; Ahmad and Winter, 1968; Marsalek *et al.*, 1992) while others have described them as Vitamin B (Grieco and Desrochers, 1978) or amino acids (Watanabe 1951; Vorontsova *et al.*, 1988), antibiotics and toxins (Metting and Pyne, 1986). Such amino acids being soluble in water may form readily available source of nitrogen for crop plants (Goyal, 1993).

These beneficial effects can be achieved only if there is good growth of BGA in rice fields. Conclusive evidence on the rate of build up of BGA biomass and its benefits can be achieved only from a long-term inoculation/incorporation study, because under tropical and subtropical conditions, most of the organic carbon fixed by BGA might be lost from the soil as a result of its rapid biochemical oxidation at the high temperatures prevailing during

the summer months of the year. Data from long-term experiments with BGA inoculation are, however, lacking (Ventura and Watanabe, 1993). Therefore, more basic studies on the effect of algalization on changes in soil properties in relation to C and N contents need to be undertaken to judge the effectiveness, viability and sustainability of BGA inoculation in rice fields.

Material and Methods

3. MATERIAL AND METHODS

Pot culture trials were conducted in rice crop under Phytotron (control) conditions in the National Phytotron Facility and under natural conditions (*kharif* season, 2002) at the National Centre for Conservation and Utilisation of Blue Green Algae (NCCUBGA), IARI, New Delhi, respectively to study the influence of algalization with blue green algal (BGA) biofertilizer strains individually in absence or in presence of different levels of nitrogen on properties of soil and productivity of rice crop.

3.1 BGA biofertilizer strains

3.1.1 Growth and maintenance

The unialgal BGA biofertilizer strains namely *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Tolypothrix tenuis* were used to study their role on C and N contribution in rice crop. These biofertilizer strains were obtained from germplasm of NCCUBGA, IARI, New Delhi. These were grown and maintained in the BG-11 medium (Stanier *et al.*, 1971) in a culture room at 28 ± 2°C temperature and 3000 Lux light intensity with 16/8 h L/D cycle. The composition of medium is provided in appendix.

Exponentially growing 14 days old culture suspension with 0.8-1.0 O.D. (at 600 nm) was used as inoculum and in each case 2% inoculum suspension was used to inoculate experimental flasks.

The identification of the strains was authenticated based upon keys given by Desikachary (1959) for morphological characteristics

and cultural behaviour. These BGA biofertilizer strains were also studied at weekly intervals, up to late log phase for different metabolic parameters. The parameters studied were growth measurements (dry weight, specific growth rate and generation time), pigment production (chlorophyll, carotenoids and phycobilins), cellular constituents (soluble proteins and total sugars) and physiological attributes (nitrogenase, nitrate reductase and glutamine synthetase activity, ammonia excretion).

3.1.2 Morphological and cultural characteristics

The following attributes were recorded for morphological characteristics through microscopic examination.

i. Size and shape of vegetative cells
ii. Heterocyst size, shape and frequency
iii. Akinete size and shape

The cultural behaviour of BGA biofertilizer strains was examined in liquid medium and agar-based medium. The colour of thallus, planktonic/benthic nature of trichomes/filaments in liquid medium and type of growth was studied.

3.1.3 Growth measurements

3.1.3.1 Dry weight (mg mL^{-1})

For determination of dry weight, algal suspension was homogenized and a known volume was filtered through a sintered glass apparatus on a preweighed Whatman No. 42 filter paper. These were then oven dried at 60°C and cooled in a desiccator until

constant weight was achieved. The difference in two weights was recorded as dry weight (Sorokin, 1973).

3.1.3.2 Specific growth rate and generation time

For the determination of specific growth rate and generation time, growth was measured in terms of absorbance of the culture suspension at 600 nm (Specord 200, Analytik Jena) on 15th and 30th day of incubation.

Specific growth rate (K) and generation time (G) was calculated by using the formula of Myers and Kratz (1955).

$$K(\text{OD/Day}) = \frac{3.32 \times (\log N_2 - \log N_1)}{T_2 - T_1}$$

$$G(\text{Days}) = \frac{T_2 - T_1}{3.32 \times (\log N_2 - \log N_1)}$$

Where, N_1 = Initial cell concentration (absorbance) at time T_1

N_2 = Final cell concentration (absorbance) at time T_2

3.1.4 Estimation of pigments

3.1.4.1 Chlorophyll

For estimation of chlorophyll, a known volume of homogenized suspension was centrifuged for 5 min (3000 × *g*). Chlorophyll from the pellet was extracted with 95% (v/v) methanol at 60°C for 30 min. After centrifugation, the final volume was made up and the chlorophyll was quantified by measuring optical density at 650 and 665 nm. Total chlorophyll was calculated according to the formula given below (Mackinney, 1941).

$$Chlorophyll(mg/mL) = 2.55 \times 10^{-2} \times E_{650} + 0.4 \times 10^{-2} \times E_{665}$$

Where, E_{650}= Absorbance at 650 nm

E_{665} = Absorbance at 665 nm

3.1.4.2 Carotenoids

A known volume of homogenized algal suspension was centrifuged at 3000 × *g* for 10 min. The pellet thus obtained was washed with distilled water to remove traces of adhering salts. To that, 2-3 mL of 85% acetone was added and subjected to repeated freezing and thawing. Extractions were performed till acetone became colourless. The acetone fractions thus obtained was pooled; and the final total volume was recorded. The content of total carotenoids was estimated from the maximum absorbance measured at 450 nm using 85% acetone as blank (Jensen, 1978).

$$Carotenoids(mg/mL) = \frac{D \times V \times F \times 10}{2500}$$

Where, D = Absorbance at 450 nm

V = Volume of the extract

F = dilution factor

It is assumed that these pigments have an average extinction coefficient of 2500.

3.1.4.3 Phycobilins

A known volume of homogenized algal suspension was centrifuged at 5000 × *g* for 10 min. and pellet was suspended in equal volume of 0.05 M phosphate buffer, pH 7.5 (obtained by mixing equal volume of 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4). Repeated freezing and thawing was done till the pellet became

colourless and the pigments oozed out in the supernatant. The absorbance was measured at 562, 615 and 652 nm against 0.05 M phosphate buffer as blank. The amounts of phycobilin pigments (Phycocyanin, PC; allophycocyanin, APC; and Phycoerythrin, PE) were calculated by using the following equations (Bennett and Bogorad, 1973).

$$Phycocyanin(mg/mL) = \frac{A_{615} - 0.474 \times A_{652}}{5.34}$$

$$Allophycocyanin(mg/mL) = \frac{A_{652} - 0.208 \times A_{615}}{5.06}$$

$$Phycoerythrin(mg/mL) = \frac{A_{562} - [12.41(PC) - 0.849(APC)]}{9.62}$$

3.1.5 Estimation of cellular constituents

3.1.5.1 Soluble proteins (μg mg^{-1} dry weight)

Reagents

A. 1 N sodium hydroxide solution

B. (i) 5% sodium carbonate

(ii) 0.5% copper sulphate ($CuSO_4$. $5H_2O$) solution in 1% sodium potassium tartarate

Two mL of reagent B (ii) was mixed with 50 mL of freshly prepared reagent B (i)

C. 1 N Folin-ciocalteau reagent

A known volume (0.5 ml) of homogenized algal cell suspension was taken in a test tube. To this, 0.5 mL of reagent (A) was added. The tubes were then heated in a boiling water bath for 10 min. and cooled in running tap water. Subsequently, 2.5 mL of reagent (B) was added in each and the tubes were incubated at

room temperature for 10 min. After this, 0.5 mL of reagent (C) was added and tubes were kept at room temperature for 15 min. The intensity of blue colour was read as absorbance at 650 nm against appropriate blank. The protein content was estimated using a standard calibration curve prepared from bovine serum albumin (Lowry *et al.*, 1951; Herbert *et al.*, 1971).

3.1.5.2 Total sugars ($\mu g\ mg^{-1}$ dry weight)

Anthrone reagent: 100 mg anthrone and 1g thiourea was dissolved in 100 mL of 75% sulphuric acid. The mixture was kept on a water bath at 85°C to dissolve the ingredients completely.

A known volume (0.25-0.5 mL) of homogenized suspension was taken in Pyrex tubes and volume was made up to one mL with distilled water. To that, 4 mL of anthrone reagent was added and tubes were transferred to boiling water bath. After 10 min., the tubes were brought to room temperature and absorbance was read at 625 nm. The sugar content was calibrated against the standard curve made with glucose (Spiro, 1966).

3.1.6 Physiological parameters

3.1.6.1 Nitrogenase activity (EC 1.18.6.1., nmol $C_2H_4\ h^{-1}$)

Nitrogenase activity was determined by acetylene reduction assay (ARA) with the help of Gas Chromatograph (Hardy *et al.*, 1973). Nitrogenase is a versatile enzyme and can reduce variety of substrates with triple bond. Hence, this enzyme can reduce acetylene to ethylene that can be measured to understand the potential of nitrogenase activity.

Running conditions for Gas Chromatograph

Detector type	:	FID, H_2 24 mL $min.^{-1}$, air 180 mL $min.^{-1}$, temperature 100°C
Column	:	Stainless steel, Length: 8', i.d. 1/8", porapak N (80/100 mesh)
Injector port	:	Temperature, 100°C
Carrier gas	:	N_2 30 mL $min.^{-1}$
Oven temperature	:	Iso-thermal, 80°C
Retention time	:	C_2H_4, 1.32 min. (relative)

Procedure

Nitrogenase activity was assayed by evaluating the ability of homogenized suspension to reduce acetylene to ethylene. Known volume (5 mL) of suspension was taken in 15 mL rimless tube, stoppered with subaseal and acetylene equal to 10% of air space (1 mL) was injected after removal of equal amount of air. The tubes were incubated for 90 min. at 28 ± 2°C under 3000 Lux light intensity. The reaction was terminated by injecting 0.1 mL of 50% TCA. The gas phase was assayed for ethylene using gas chromatograph (GC 1000, Chemito model) calibrated for standard ethylene.

3.1.6.2 Nitrate reductase (NR) activity

(EC 1.6.6.1., μmol NO^-_2 μg^{-1} protein)

Nitrate reductase is a substrate inducible enzyme and its activity is based upon its ability to reduce nitrate to nitrite that is measured by diazocoupling method.

Reagents

(A) 1% sulphanilamide in 100 mL of 1N HCl.

(B) 0.2% NEDD (N-1-naphthyl ethylene diamine dichloride) in 100 mL of distilled water.

The blue green algal cultures were incubated in BG-11 medium supplemented with 10 mM $NaNO_3$ for 12-16 hrs to induce nitrate reductase activity. One mL of such induced sample was taken in a test tube, to which 2 mL of reagent A was added and incubated for 15 min. After that, 2 mL of reagent B was added and pink colour was allowed to develop for 15 min. Absorbance was recorded at 540 nm and the amount of nitrite was estimated from standard curve of $NaNO_2$ (Lowe and Evans, 1964).

3.1.6.3 Glutamine synthetase (GS) activity (EC 6.3.1.2., μmol γ-glutamyl hydroxamate μg^{-1} protein 30 $min.^{-1}$)

The transferase activity of GS was determined at pH 7.0 by measuring the amount of γ-glutamyl hydroxamate formed using glutamine as substrate in presence of ADP and arsenate. Whole cell GS activity was analysed after toluene treatment of cells. One unit of enzyme activity is defined as the amount of enzyme needed to form 1 μmol of product per min. at 37°C under optimum conditions.

Reagents

A. Extraction buffer: Prepared by dissolving 5 mM $MgCl_2$ and 10 mM sodium glutamate in 50 mM imidazole-HCl solution (pH 7.0).

B. Assay mixture: Prepared by dissolving manganese chloride 0.1 M, glutamine 0.1 M (pH 7.0), disodium hydrogen arsenate 1.0 M (pH 7.0), ADP (sodium salt) 0.01 M (pH 7.0), hydroxylamine hydrochloride 2.0 M, sodium hydroxide 2.0 N in 50 mM imidazole-HCl buffer (pH 7.0).

C. Stop mixture: Prepared by mixing 4 mL ferric chloride (10%), 1.0 mL TCA (24%), 0.5 mL 6 N HCl and 6.5 mL double distilled water.

A known volume of homogenized suspension was centrifuged at 5000 × *g* for 5 min. To the pellet, 0.25 mL of toulene was added and incubated for 10 min. at 4°C. During incubation, the tubes were repeatedly shaken to ensure complete permeability of cell membranes and release of the enzyme. After centrifugation, the toluene layer was discarded and 0.8 mL of extraction buffer and 1 mL of assay mixture were added. The suspension was incubated at 37°C for 30 min. and the reaction was terminated by adding 2 mL of stop mixture. The absorbance was read at 540 nm and the activity was directly read from standard curve of γ-glutamyl hydroxamate (Shapiro and Stadtman, 1970; Stacey *et al.*, 1977).

3.1.6.4 Ammonia excretion (mmol NH^+_4 mL^{-1})

The amount of ammonia in culture filtrates (extracellular release of ammonia) was assayed by employing the Phenol-Hypochlorite method.

Reagents

(A) 10% phenol dissolved in 95% ethanol

(B) 0.5% sodium nitroprusside

(C) 100 mL of alkaline reagent (100 g trisodium citrate and 5 g sodium hydroxide dissolved in 500 mL distilled water) with 25 mL sodium hypochlorite solution (4% w/v)

A known volume of blue green algal suspension was taken and centrifuged at 3000 × *g* for 10 min. To 5 mL of culture filtrate, 0.2 mL each of reagent A and B was added followed by addition of 0.5 mL reagent C. After thorough mixing, the colour was allowed to develop for 1 h. The absorbance was read at 640 nm. The amount of ammonia excreted was directly read from standard curve of NH_4Cl (Solorzano, 1969).

3.2 Crop, variety and growth conditions

Soil and plant parameters were analysed in pot culture experiments in rice crop to evaluate the role of blue green algal biofertilizer strains on carbon and nitrogen contribution. The test crop utilized for present study was high fertilizer responsive scented rice variety Basmati-1. This variety is one among the preferred scented rice varieties found to perform well under North Indian

conditions. This is a long duration variety (160 days) with a yield potential ranging from 3 to 4 tonnes ha^{-1}.

Under Phytotron conditions, experiment was conducted using plastic pots, size 8" × 8" (h × d), filled with peat as an inert base for raising rice crop at National Phytotron Facility, IARI. The experimental facility provided the suitable growing conditions (32°C day temperature and 28°C night temperature) for rice crop. The physico-chemical characteristics of peat used for the raising crop are mentioned below.

Physicochemical properties of peat

Bulk density (g cm^{-3})	1.04
Particle density (g cm^{-3})	2.12
Water holding capacity (%)	130
pH	6.10
Organic carbon (%)	24.28

Under natural conditions, the experiment was conducted at NCCUBGA, IARI in earthen pots, size 12" × 14" (h × d), filled with 8 kg unsterilized soils. Soil having sandy loam texture (United States Soil Survey, 1951) was collected from the experimental farm (MB-8B Block) of Indian Agricultural Research Institute, New Delhi. The collected soil was mixed thoroughly, air-dried and passed through 4 mm sieve. The soil, which belonged to Typic Ustochrept was analysed for its physico-chemical and biological properties (Details given as under).

A. Mechanical composition (Bouyoucos, 1962)

Sand	67.5%
Silt	18.2%
Clay	14.3%

B. Chemical composition

Organic carbon (%)	0.43
Available N (kg ha^{-1})	208
pH	7.67
EC (dS m^{-1})	1.25

C. Biological properties

Soil dehydrogenase activity (µl H_2 g^{-1} day^{-1})	1.16
Microbial biomass carbon (mg kg^{-1})	0.885
Soil chlorophyll (µg g^{-1})	0.817
Soil ARA (nmol C_2H_4 g^{-1} h^{-1})	0.008

3.3 Experimental details

The experiments were conducted in complete randomized block design (C.R.D) under Phytotron as well as under natural conditions to examine the effect of algalization with BGA biofertilizer strains namely *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Tolypothrix tenuis*. Biofertilizer strains were used individually in the absence and in the presence of inorganic N levels. The treatment combination N_0S_0 (no nitrogen, uninoculated condition) served as control. Treatments with N_1 (50% of recommended dose) and N_2

(recommended dose, 100%) levels of nitrogenous fertilizer were also included without BGA inoculation in the present study.

Thus, the following treatment combinations with three replications were used to investigate the role of BGA strains on C and N contribution in rice crop.

N levels

1. N_0 : No nitrogen
2. N_1 : 50% of recommended N rate
3. N_2 : Recommended rate *i.e.*, 100% N

BGA strains

1. S_0 : Without BGA (uninoculated control)
2. S_1 : *Anabaena variabilis*
3. S_2 : *Aulosira fertilissima*
4. S_3 : *Nostoc muscorum*
5. S_4 : *Tolypothrix tenuis*

Two sets of treatment combinations were maintained for the purpose of sampling during midcrop stage (MS) and at the time of harvest (HS). Hence, in all, there were 15 × 3 × 2 *i.e.*, 90 pots used for the present study.

Under Phytotron conditions, seedlings were raised in specialized plastic trays (Hugo's trays) containing 64 cup like cavities. These cavities were filled with peat as a rooting media and seeds were sown in them (4 seeds per cavity) in the glass house facility. These trays were initially watered with sterilized distilled water for a week and subsequently irrigated with standard Hoagland solution (Hoagland, 1944). The composition of

Hoagland's solution is given in appendix. For the pot culture experiment conducted under natural conditions, seedlings were provided by Seed Production Unit, IARI, New Delhi, raised in field during *kharif* 2002 following the standard procedure. Each pot was planted with 25 days old, 3 seedlings/pot under Phytotron conditions and 5 seedlings/pot under natural conditions.

Under Phytotron conditions, the specified pots were inoculated with unialgal suspension of BGA biofertilizer strains (equivalent to 250 μg chl pot^{-1}) after one week of transplantation. The pots were irrigated with modified Hoagland solution, which was standardized for the nutrients essential for growth of rice. The nitrogen concentration was adjusted with ammonium nitrate *i.e.*, @ 0% N, 50% N and 100% N (as per the composition of Hoagland's solution). However, the concentration and composition of other salts were adjusted by modifying the nutrient recipe (composition details provided in appendix). The pots were daily irrigated with modified Hoagland solution and were flushed with distilled water once in a fortnight to remove the toxic effects of salts that might have accumulated in the pots.

Under natural conditions, nitrogen was applied as urea @ 60 kg N ha^{-1} and 120 kg N ha^{-1} in three splits as basal dose, at 30 days and 60 days after transplanting. A set of pots for N_0 treatment did not receive urea as the source of nitrogen. Phosphorus was applied as basal dressing in the form of single super phosphate (SSP) @ 50 kg P_2O_5 ha^{-1} and potassium was applied as muriate of potash @ 50 kg K_2O ha^{-1} in two splits at basal and 60 days after transplanting in all the

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pots. Crop was inoculated with suspension of unialgal BGA biofertilizer strains (equivalent to 250 μg chl pot^{-1}) after one week of transplantation in specific pots as outlined in the treatment schedule. The additions were thoroughly mixed and the level of water was maintained at 2.5 cm during early stage and 5 cm of water in later stages of the crop growth up to 15 days before harvest.

In the inoculated pots, both under Phytotron as well as natural conditions, blue green algal growth was observed as a thick leathery sheet on the surface.

3.4 Analysis

3.4.1 Peat

Under Phytotron conditions, peat was used in pots for raising crop (Peat was carefully scooped out from the experimental pots during midcrop and at harvest time of crop growth). Assuming peat to be an inert material, only BGA abundance (CFU g^{-1}, as per procedure No. 3.4.2.1 for BGA) was analysed.

3.4.2 Soil

The soil surface layer of 1.5 cm was scooped out using sharp spatula at two stages of crop growth *i.e.*, during mid crop growth and at the time of harvest. These collected soil samples were dried in shade and passed through a 2 mm sieve and were used for further analysis. Samples of about 100 g were retained for the analysis.

These samples were analysed for BGA abundance, bacteria, fungi, and actinomycetes populations, pH, EC, redox potential, chlorophyll, dehydrogenase activity (a non-specific measure of soil

metabolic activity), nitrogenase activity (to assess the activity of diazotrophs) and microbial biomass carbon in order to understand the effect of blue green algal inoculation on soil microbiological and biochemical properties (Rao and Burns, 1990). The soils were also analysed to quantify C and N productivity levels.

3.4.2.1 Microbial flora (CFU g^{-1} soil)

The microbial populations (BGA, bacteria, fungi and actinomycetes) were assessed in the soil through standard serial dilution plating technique. For the determination of blue green algal abundance, BG-11 medium was used (Stanier *et al.*, 1971, appendix). The incidence of BGA was quantified as CFU per gram of soil using standard pour plate method (Roger *et al.*, 1991). Incubation was done at 28 ± 2°C, 3000 Lux light intensity and 16:8 h L/D cycle. The plates were regularly observed and data regarding BGA population was recorded. Similarly, soil extract agar medium for bacteria (Parkinson *et al.*, 1971, appendix), Martin's Rose Bengal agar medium for fungi (Martin, 1950, details of medium in appendix) and starch casein medium for actinomycetes (Kuster and Williams, 1964, details of medium in appendix) were used for enumeration of other soil micro flora.

3.4.2.2 pH and EC (dS m^{-1})

Soil pH and EC were estimated in a soil water suspension of 1:2 ratio (Jackson, 1973). EC was measured using a conductivity meter (Model CM-185, Elico, India) and pH was recorded using electric pH meter (Model LI-120, Elico, India).

3.4.2.3 Redox potential (mV)

The redox potential (Eh) of the soil sample was measured with a portable redox meter (Model LI-120, Elico, India) fitted with a compound platinum and calomel electrode. Before taking readings, a portion of the top oxidized layer was scooped out carefully and electrode was placed in the reduced zone. The potentials (mV) are based on the standard hydrogen electrode by adding +245 mV to redox readings (Saha *et al.*, 1982).

3.4.2.4 Soil chlorophyll (mg L^{-1})

Soil chlorophyll was extracted within several hours after the sampling (Tsujimura *et al.*, 2000). Preweighed soil cores were mixed with acetone: DiMethyl Sulphoxide (DMSO) mixture in 1:10 ratio @ 4 mL g^{-1} soil. After shaking thoroughly, these were left for 24 h in the dark at room temperature. Shaking was repeated several times for the complete extraction of chlorophyll from the soil samples and the concentrations were determined from absorbance readings taken at 663, 645 and 630 nm using the equation described below.

$$Chlorophyll(mg/mL) = 11.64 \times OD_{663} - 2.16 \times OD_{645} + 0.10 \times OD_{630}$$

3.4.2.5 Dehydrogenase activity (µl H_2 released g^{-1} soil day^{-1})

The enzyme activity in soil was determined by 2,3,5-triphenyl tetrazolium chloride (TTC) reduction method (Klein *et al.*, 1971). Representative soil samples (1 g) were taken in air–tight screw capped

test tubes. To each tube 0.2 mL of 3% solution of TTC was added followed by 0.5 mL of 1% glucose solution. These tubes were then incubated in the dark at room temperature for 24 h. The triphenyl formazon (TPF) formed due to dehydrogenase activity of microbes was extracted with methanol and assayed by measuring its absorbance at 485 nm. Standard calibration curve was prepared from triphenyl formazon and the activity was expressed as µl H_2 released g^{-1} soil day^{-1}.

3.4.2.6 Nitrogenase activity (nmol C_2H_4 h^{-1} g^{-1} soil)

Soil nitrogenase activity was examined by acetylene reduction assay (ARA). Preweighed soil cores were taken in glass vials and stoppered with subaseal. 10% of air was removed from the gas phase of the vial and equal amount of acetylene was injected. After that, vials were incubated for 3 h outside under natural conditions. After incubation, gas phase was examined for ethylene by gas chromatography using porapak N column (GC 1000, Chemito model). Control with soil cores without acetylene was also included. C_2H_4 production was not detectable in the absence of C_2H_2 (Prasanna *et al.*, 2003).

3.4.2.7 Microbial biomass carbon (mg kg^{-1} soil)

Microbial biomass carbon was measured by the chloroform fumigation-extraction method. Weighed soil samples (35 g, each replication) were taken in 250 mL Schott's bottle and fumigated with ethanol-free chloroform for 24 h. After this, chloroform was removed through standard procedure and the subsequent extraction of soil was

done with 140 mL of 0.5 M K_2SO_4 for 30 min. The unfumigated (without chloroform) control soil was extracted in the similar manner. The extract was filtered through Whatman No. 42 filter paper and O.D. was taken at 280 nm and results were expressed as mg kg^{-1} soil (Page *et al.*, 1982).

3.4.2.8 Organic carbon (%)

Oxidizable C was estimated by wet digestion method (Walkley and Black, 1934; Piper, 1966).

3.4.2.9 Available Nitrogen (kg N ha^{-1})

This was estimated by the alkaline $KMnO_4$ method for all sets of samples (Subbiah and Asija, 1956).

3.4.3 Plants

Under Phytotron as well as natural conditions, plants from replicated pots were uprooted and pooled for dry matter determinations during mid crop and at harvest time of crop growth. The dried plant samples were ground into fine powder and used for further analysis regarding carbon content and nitrogen uptake.

3.4.3.1 Dry matter (g pot^{-1})

For dry weight analysis, the representative plant samples (root and shoot and grains at harvest) were kept in oven at 60°C till constant weight was achieved. Dry matter production for the plants was expressed on per pot basis.

3.4.3.2 Carbon content (g pot^{-1})

This was estimated by chromic acid oxidation method in representative powdered plant samples (Jackson, 1973).

3.4.3.3 Total nitrogen (mg pot^{-1})

The N uptake by plant samples (shoot, root and grains) was estimated separately by a modified microkjeldahl method (Humphries, 1956).

3.4.3.4 Yield attributes

Data on yield attributes like panicle number pot^{-1}, grain number $panicle^{-1}$, 1000 grain weight (g) and grain yield (g pot^{-1}) were also recorded at the harvest time.

3.5 Statistical evaluation

The data recorded on various parameters were subjected to statistical analyses using M-STATC package according to factorial CRD. Duncan's Multiple Range Test (DMRT) was employed to compare the mean performances of strains for specific parameters. This facilitated the identification of superior treatment.

Results

4. RESULTS

The present investigation conducted at National Phytotron Facility and NCCUBGA (during *kharif* season, 2002), IARI, New Delhi, aimed to examine the influence of blue green algal biofertilizer strains on certain specific soil properties in relation to carbon and nitrogen contribution and their impact on productivity of rice crop under various nitrogen levels.

4.1 BGA biofertilizer strains

Comparative morphological and cultural characteristics, growth potential, pigment production, cellular constituents and physiological parameters were analysed in unialgal blue green algal biofertilizer strains namely *Anabaena variabilis*, *Aulosira fertilissima*, *Nostoc muscorum* and *Tolypothrix tenuis*.

4.1.1 Growth and maintenance

The unialgal BGA biofertilizer strains were grown, regularly subcultured and maintained in BG-11 medium (both liquid and on agar slants) in a culture room at 28 ± 2°C temperature and 3000 Lux light intensity with 16/8 h L/D cycle. For each analysis, exponentially growing 14 days old culture suspension was used as inoculum and observations were recorded till late log phase.

4.1.2 Morphological and cultural characteristics

The morphological and cultural characteristics of blue green algal biofertilizer strains were examined during exponential growth in

nitrogen free BG-11 medium. These strains were studied microscopically in terms of size and shape of vegetative cells, heterocysts and akinetes; position of heterocysts in filaments and their frequency. From these parameters, the identification and purity of the biofertilizer strains were confirmed as per the keys given by Desikachary (1959) (Table 1.1, Plates 2a, b, c and d).

Analysis of cultural behaviour carried out in liquid and agar based solid media indicated that all the forms were planktonic in nature except *Aulosira fertilissima* which exhibited benthic behaviour. Colour of the thalli ranged from dark green, blue green to brown. In solid media, these organisms showed globose, spreading, mucilaginous or pin-head colonies whereas in liquid media these expressed spreading thread like mucilaginous growth. Other parameters studied included trichome form and the nature of the trichome which varied in the strains being investigated (Table 1.2).

4.1.3 Growth measurements

4.1.3.1 Dry weight (mg mL^{-1})

Mean dry weight calibrated during the incubation time till late log phase varied from 1.28 mg mL^{-1} in *Anabaena variabilis* to 0.91 mg mL^{-1} in *Aulosira fertilissima*. Mean dry weight for *Nostoc muscorum* and *Tolypothrix tenuis* was 1.15 mg mL^{-1} and 0.95 mg mL^{-1} respectively. All other parameters examined were mostly expressed on unit dry weight basis.

Table 1. Characteristics of blue green algal biofertilizer strains

1.1 Morphological characteristics of biofertilizer strains

Strains	Vegetative cell			Heterocyst					Akinete		
	Shape	Size (μ)		Shape	Size (μ)		Position*	Frequency (%)	Shape	Size (μ)	
		Length	Breadth		Length	Breadth				Length	Breadth
S_1	Barrel	5.7	3.8	Barrel	7.6	5.7	I&T	14.28	Cylindrical	11.4	7.6
S_2	Elongated	7.0	15.2	Oblong	19.2	11.4	I	9.1	Oblong	17.4	11.4
S_3	Barrel	11.2	11.2	Spherical	7.6	7.6	I	6.6	Oblong	11.2	7.6
S_4	Broad	6.0	5.7	Round	7.6	3.8	I&T	7.6	Elliptical	17.4	7.6

* I&T denotes intercalary and terminal

1.2 Cultural characteristics of biofertilizer strains

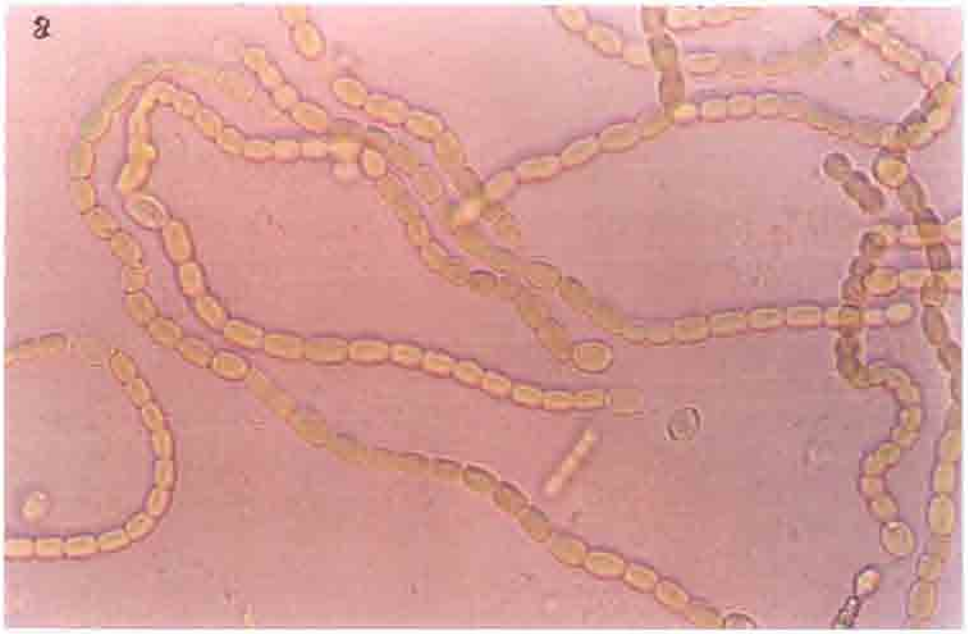
Strains	Life form	Colour of Thallus	Pattern of growth		Trichome form	Nature of trichome	Specific Growth rate (OD/day)	Generation time (Days)
			In solid medium	In liquid medium				
S_1	Planktonic	Dark green	Globose	Spreading	Straight	Trichome constricted, without sheath or branch, motile	0.487	2.053
S_2	Benthic	Blue green	Spreading	Thread like	Straight	Trichome with sheath, pseudo branch	0.430	2.326
S_3	Planktonic	Dark green	Mucilaginous	Mucilaginous	Beaded entangled	Trichome without sheath or branch, sedentary	0.301	3.322
S_4	Planktonic	Brown	Pin head colony	Spreading	Straight	Trichome with sheath, branched	0.253	3.953

S_1 =*Anabaena variabilis*; S_2 =*Aulosira fertilissima*; S_3 =*Nostoc muscorum*; S_4 =*Tolypothrix tenuis*

Plate 2. Photomicrographs of BGA biofertilizer strains (Scale bars = 10 μ)

a) ***Anabaena variabilis*** **(720X)**

b) Aulosira fertilissima **(562.5X)**


a

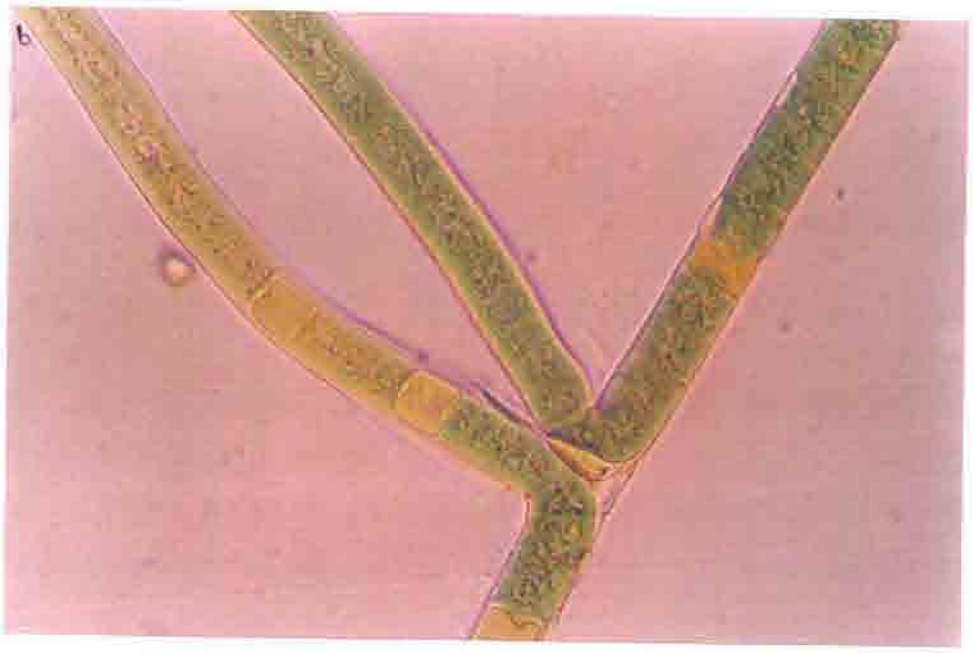
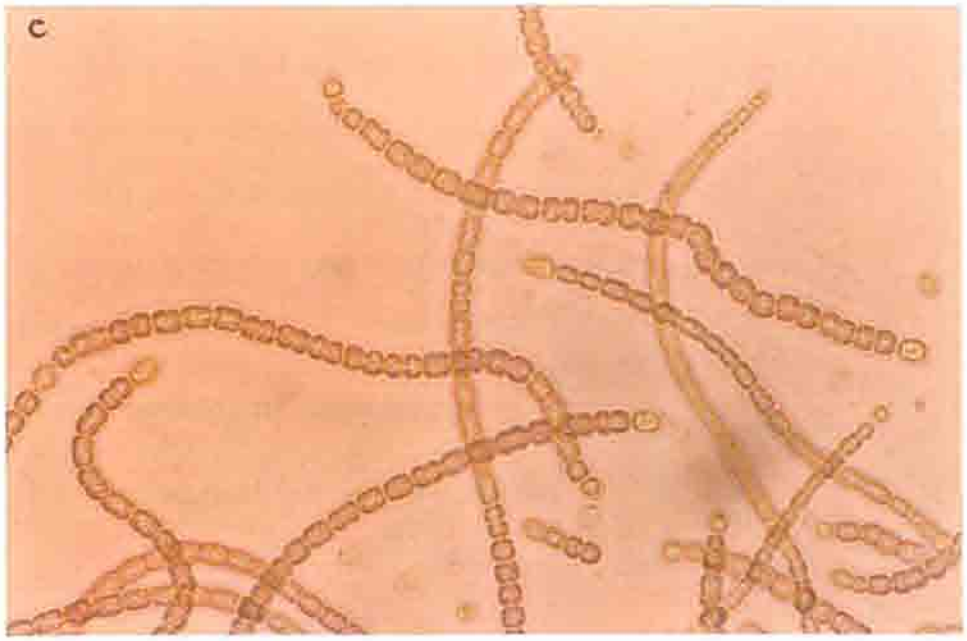

b

Plate 2. Photomicrographs of BGA biofertilizer strains (Scale bars = 10 μ)

c) *Nostoc muscorum* (450X)

***d) Tolypothrix tenuis* (450X)**


c


d

4.1.3.2 Specific growth rate (OD/Day) and generation time (Days)

Specific growth rate and generation time were studied on the basis of O.D. measurements during 15th and 30th day of incubation. Specific growth rate was highest in *Anabaena variabilis* and lowest in *Tolypothrix tenuis* with the generation time being maximum in *Tolypothrix tenuis* (Table 1.2).

4.1.4 Estimation of pigments

4.1.4.1 Chlorophyll (μg mg^{-1} dry weight)

Overall mean calculated for chlorophyll content during incubation time till late log phase exhibited peak value at 7th day of growth followed by a gradual decline. There was a significant difference among the strains for this parameter and the chlorophyll content ranged between 5.25 μg mg^{-1} dry weight in *Tolypothrix tenuis* and 8.42 μg mg^{-1} dry weight shown by *Aulosira fertilissima*. Based upon mean performance for strains × days of incubation interaction using DMRT analysis, it was seen that *Anabaena variabilis* showed highest chlorophyll content at 7th day of incubation followed by its chlorophyll content at 14th day of incubation and by *Nostoc muscorum* at 7th day of incubation. *Aulosira fertilissima* exhibited almost similar chlorophyll content at 7th and 14th day of incubation (Table 2).

4.1.4.2 Carotenoids (μg mg^{-1} dry weight)

Mean values of carotenoids calculated were highest during peak growth stage (i.e. 14th day of incubation) followed by a decline

Table 2. Chlorophyll content ($\mu g\ mg^{-1}$ dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.353^{l}	0.254^{l}	0.26^{l}	0.332^{l}	0.300
7	17.42^{a}	13.32^{bc}	14.16^{b}	8.76^{ef}	13.41
14	14.64^{b}	12.58^{bc}	8.20^{efg}	6.07^{ghij}	10.37
21	9.69^{de}	11.31^{cd}	7.19^{fgh}	5.42^{hijk}	8.40
28	6.69^{fghi}	12.64^{bc}	5.56^{ghijk}	4.39^{ijk}	7.32
35	3.55^{jk}	6.47^{fghi}	4.91^{hijk}	5.18^{hijk}	5.03
42	3.64^{jk}	6.21^{fghij}	6.41^{fghi}	6.21^{fghij}	5.62
49	3.01^{k}	4.61^{hijk}	5.68^{ghijk}	$5.62g^{hijk}$	4.73
Mean	7.37	8.42	6.55	5.25	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.283		0.784	
Days (D)		0.401		1.111	
S × D		0.801		2.219	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

thereafter. There was a marked difference amongst the biofertilizer strains for this parameter. *Tolypothrix tenuis* showed highest carotenoid content (0.338 μg mg^{-1} dry weight) and *Aulosira fertilissima* showed lowest carotenoid content (0.108 μg mg^{-1} dry weight). DMRT analysis carried out for strains × incubation time revealed that *Tolypothrix tenuis* exhibited highest carotenoids at 14^{th} day of incubation followed by carotenoid content at 7^{th} day of incubation by the same strain. Carotenoid content at 14^{th} day by *Anabaena variabilis* assumed third position (Table 3).

4.1.4.3 Phycobilins (μg mg^{-1} dry weight)

Phycobilins comprising phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) were measured individually in BGA biofertilizer strains grown in nitrogen free BG-11 medium during the incubation time. Highest phycocyanin, allophycocyanin and phycoerythrin contents were observed at 14^{th} day of incubation. *Nostoc muscorum* showed highest phycocyanin and allophycocyanin. *Aulosira fertilissima* showed lowest phycocyanin and allophycocyanin. Phycoerythrin was highest in *Tolypothrix tenuis*, which was almost similar to the value observed for *Nostoc muscorum* and lowest in *Aulosira fertilissima*. Interaction studies for blue green algal strains and days of incubation for phycocyanin content have indicated that *Tolypothrix tenuis* at 14^{th} day of incubation revealed a top grouping combination followed by phycocyanin content exhibited by *Anabaena variabilis* at similar time of incubation. An almost similar trend was shown for allophycocyanin content by these strains examined.

Table 3. Carotenoid content ($\mu g\ mg^{-1}$ dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.024^{ij}	0.031^{ij}	0.025^{ij}	0.029^{ij}	0.027
7	0.370^{cd}	0.144^{ghi}	0.237^{efg}	0.720^{b}	0.368
14	0.411^{c}	0.278^{def}	0.246^{efg}	0.911^{a}	0.462
21	0.033^{ij}	0.117^{hij}	0.168^{fgh}	0.290^{de}	0.152
28	0.017^{j}	0.050^{ij}	0.116^{hij}	0.055^{hij}	0.060
35	0.016^{j}	0.028^{ij}	0.039^{ij}	0.021^{ij}	0.026
Mean	0.146	0.108	0.139	0.338	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.015		0.042	
Days (D)		0.018		0.050	
S × D		0.036		0.100	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

Phycoerythrin was also highest for *Tolypothrix tenuis* followed by *Nostoc muscorum* at 14th day of incubation (Tables 4.1,4.2,4.3).

4.1.5 Estimation of cellular constituents

4.1.5.1 Soluble proteins (μg mg^{-1} dry weight)

Soluble proteins analysed were highest at 14th day of incubation i.e., during the active growth phase of the blue green algal strains. This parameter followed a typical exponential pattern resulting in a slow and gradual decrease towards late log phase. The strains differed significantly with respect to this parameter and *Aulosira fertilissima* showed the highest soluble protein content (46.04 μg mg^{-1} dry weight) and *Nostoc muscorum* showed lowest value (28.23 μg mg^{-1} dry weight). Strains × days of incubation analysis was significant at all stages. Results based upon DMRT analysis revealed that *Aulosira fertilissima* showed highest soluble protein at 14th day of incubation followed by the amount recorded at 21st day of incubation, which was *at par* with soluble protein content shown by *Anabaena variabilis* at 14th day of incubation. The soluble protein content expressed by *Aulosira fertilissima* at 7th day of incubation and *Nostoc muscorum* at 14th day of incubation was third in the order (Table 5).

4.1.5.2 Total sugars (μg mg^{-1} dry weight)

The total sugars analysed showed a linear enhancement with incubation time, a maximum mean value exhibited at 21st day of incubation followed by a gradual and linear decline. *Aulosira*

Table 4. Phycobilin content of biofertilizer strains

4.1 Phycocyanin content ($\mu g\ mg^{-1}$ dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.027
7	5.87^{de}	2.02^{e}	11.49^{d}	0.61^{e}	0.368
14	38.35^{b}	5.37^{de}	23.49^{c}	47.02^{a}	0.462
21	6.05^{de}	3.20^{e}	19.87^{c}	2.24^{e}	0.152
28	2.51^{e}	0.53^{e}	0.33^{e}	0.05^{e}	0.060
35	0.99^{e}	1.57^{e}	0.85^{e}	3.58^{e}	0.026
Mean	8.96	2.11	9.34	8.92	

	SE (m)±	CD (P=0.05)
Strains (S)	0.027	0.073
Days (D)	0.368	0.994
S × D	0.462	1.250

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

4.2 Allophycocyanin content ($\mu g\ mg^{-1}$ dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.00^{i}	0.00^{j}	0.00^{j}	0.00^{j}	0.00
7	2.17^{fghij}	1.91^{ghij}	4.28^{def}	3.42^{defg}	2.95
14	11.42^{b}	5.51^{d}	8.88^{c}	25.09^{a}	12.72
21	1.80^{ghij}	2.20^{fghij}	5.09^{de}	2.55^{fghi}	2.91
28	0.77^{hij}	0.33^{ij}	0.62^{hij}	0.00^{j}	0.43
35	1.02^{ghij}	1.90^{ghij}	1.05^{ghij}	2.93^{efgh}	1.72
Mean	2.86	1.97	8.32	5.66	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.296		0.799	
Days (D)		0.362		0.977	
S × D		0.725		1.958	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

4.3 Phycoerythrin content ($\mu g\ mg^{-1}$ dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.00^{i}	0.00^{i}	0.00^{i}	0.00^{i}	0.00
7	4.99^{fgh}	2.31^{fghi}	9.74^{e}	6.02^{f}	5.77
14	33.84^{c}	5.33^{fgh}	38.03^{b}	56.67^{a}	33.47
21	3.42^{fghi}	2.75^{fghi}	17.86^{d}	2.59^{fghi}	6.66
28	2.17^{ghi}	0.57^{i}	0.32^{i}	0.01^{i}	0.77
35	0.86^{i}	1.56^{hi}	0.86^{i}	5.47^{fg}	2.19
Mean	7.55	2.09	11.14	11.79	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.470		1.27	
Days (D)		0.576		1.56	
S × D		1.152		3.11	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

Table 5. Soluble proteins (μg mg^{-1} dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	1.46^{o}	1.34^{o}	1.28^{o}	1.27^{o}	1.34
7	52.16^{fg}	67.51^{c}	48.04^{g}	62.27^{d}	57.50
14	75.67^{b}	102.16^{a}	69.91^{c}	55.68^{ef}	75.85
21	58.93^{de}	77.73^{b}	37.27^{h}	40.94^{h}	53.72
28	28.86^{i}	39.96^{h}	20.37^{lm}	26.62^{ijk}	28.95
35	27.99^{ij}	40.31^{h}	20.86^{lm}	25.41^{ijkl}	28.64
42	21.60^{klm}	27.61^{ij}	16.93^{m}	23.19^{ikl}	22.33
49	10.35^{n}	11.67^{m}	11.21^{n}	16.42^{m}	12.41
Mean	34.63	46.04	28.23	31.48	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.590		1.634	
Days (D)		0.835		2.313	
S × D		1.670		4.626	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

fertilissima showed highest mean value of 99.0 μg mg^{-1} dry weight and *Anabaena variabilis* showed lowest mean value (77.4 μg mg^{-1} dry weight). The interaction analysis (strains × days of incubation) carried out was significant and indicated that *Aulosira fertilissima* and *Nostoc muscorum* showed highest amount of total sugars followed by *Tolypothrix tenuis* at 21st day of incubation and *Nostoc muscorum* at 14th day of incubation (Table 6).

4.1.6 Physiological parameters

4.1.6.1 Nitrogenase activity (EC 1.18.6.1., nmol C_2H_4 h^{-1})

Nitrogenase activity calculated on the basis of per mg of dry weight, per mg of chlorophyll and per μg of protein exhibited more or less similar behaviour by blue green algal strains examined during the incubation time. Highest nitrogenase activity was observed at 14th day of incubation in all the three cases, followed by a gradual decline. There was a marked difference observed in different biofertilizer strains with respect to this attribute. On unit dry weight basis, *Aulosira fertilissima* showed highest (29.96 nmol C_2H_4 mg^{-1} dry weight h^{-1}) nitrogenase activity and *Tolypothrix tenuis* exhibited lowest activity (17.68 nmol C_2H_4 mg^{-1} dry weight h^{-1}). The top three groups calculated on the basis of DMRT analysis were *Nostoc muscorum* and *Aulosira fertilissima* at 14th day of growth and the later strain at 7th day of incubation (Table 7.1).

On chlorophyll basis, *Nostoc muscorum* exhibited highest activity (1548 nmol C_2H_4 mg^{-1} chl h^{-1}) followed by *Tolypothrix tenuis* (1384 nmol C_2H_4 mg^{-1} chl h^{-1}). *Anabaena variabilis* showed

Table 6. Total sugars ($\mu g\ mg^{-1}$ dry weight) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	1.10^{n}	0.96^{n}	1.1^{n}	0.77^{n}	0.98
7	68.40^{jk}	102.3^{ghi}	100.2^{ghi}	103.3^{ghi}	93.56
14	124.5^{efg}	140.8^{de}	169.6^{bc}	151.4^{cd}	146.6
21	135.4^{def}	213.9^{a}	212.6^{a}	191.0^{ab}	188.2
28	115.9^{fgh}	105.9^{ghi}	95.6^{hi}	84.8^{ij}	100.6
35	83.7^{ij}	134.5^{def}	85.5^{ij}	108.3^{ghi}	103.0
42	68.8^{jk}	61.9^{jk}	46.8^{kl}	49.2^{kl}	56.7
49	21.7^{mn}	32.1^{lm}	47.3^{kl}	26.3^{lm}	31.9
Mean	77.4	99.0	94.8	89.4	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		2.787		7.720	
Days (D)		3.941		10.917	
S × D		7.883		21.840	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

Table 7 Nitrogenase activity of biofertilizer strains

7.1 Nitrogenase activity (ARA – nmol C_2H_4 mg^{-1} dry weight h^{-1}) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00
7	90.55^{d}	96.18^{c}	59.28^{g}	42.76^{h}	72.19
14	87.62^{e}	100.42^{b}	119.82^{a}	75.29^{f}	95.79
21	36.38^{i}	30.49^{j}	21.97^{k}	14.55^{l}	25.85
28	6.58^{m}	6.74^{m}	6.37^{m}	4.05^{mnop}	5.94
35	4.15^{mno}	4.72^{mno}	5.59^{mn}	3.51^{mnopq}	4.49
42	1.76^{opqr}	0.62^{qr}	2.75^{nopqr}	0.87^{pqr}	1.50
49	0.44^{qr}	0.52^{qr}	0.41^{qr}	0.38^{qr}	0.44
Mean	28.43	29.96	27.02	17.68	

	SE (m)±	CD (P=0.05)
Strains (S)	0.359	0.994
Days (D)	0.508	1.407
S × D	1.015	2.812

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

lowest activity (1136 nmol C_2H_4 mg^{-1} chl h^{-1}). DMRT analysis carried out for strains × days of incubation showed that *Nostoc muscorum, Tolypothrix tenuis* and *Aulosira fertilissima* were grouped at top three levels at 14th day of incubation (Table 7.2).

Mean activity calculated on per unit protein basis differed significantly for the strains examined with the highest value shown by *Nostoc muscorum* (0.871 nmol C_2H_4 μg^{-1} protein h^{-1}) and lowest activity was exhibited by *Tolypothrix tenuis* (0.569 nmol C_2H_4 μg^{-1} protein h^{-1}), which was *at par* with the activity observed for *Aulosira fertilissima* (0.572 nmol C_2H_4 μg^{-1} protein h^{-1}). DMRT analysis indicated that *Nostoc muscorum* showed highest activity at 14th day of incubation followed by *Tolypothrix tenuis* and *Anabaena variabilis* at same time of incubation (Table 7.3).

4.1.6.2 Nitrate reductase (NR) activity (EC 1.6.6.1., μmol NO^-_2 μg^{-1} protein)

Nitrate reductase enzyme is an important parameter to analyze the N assimilatory capacity by the routinely used biofertilizer strains in paddy cultivation. Strains differed significantly with respect to this parameter and *Aulosira fertilissima* showed highest NR activity (70.96 μmol NO^-_2 μg^{-1} protein) whereas *Anabaena variabilis* showed lowest (41.37 μmoles NO^-_2 μg^{-1} protein) activity. Peak in nitrate reductase activity was at 14th day of incubation followed by slow and gradual decrease in the activity. Interaction studies undertaken based upon DMRT analysis showed that *Aulosira fertilissima* expressed highest nitrate reductase activity

7.2 Nitrogenase activity (ARA– nmol C_2H_4 mg^{-1} chl h^{-1}) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0^{o}	0^{o}	0^{o}	0^{o}	0
7	2598^{f}	3611^{d}	2092^{g}	2441^{f}	2686
14	2993^{e}	3991^{c}	7309^{a}	6199^{b}	5123
21	1876^{h}	1348^{i}	1528^{i}	1342^{i}	1523
28	795^{j}	412^{lm}	655^{jk}	648^{jk}	627
35	617^{jk}	389^{l}	583^{kl}	359^{mn}	487
42	141^{o}	30^{o}	184^{no}	47^{o}	100
49	73^{o}	57^{o}	36^{o}	33^{o}	49
Mean	1136	1229	1548	1384	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		22.65		62.74	
Days (D)		32.03		88.72	
S × D		64.06		177.4	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

7.3 Nitrogenase activity (ARA – nmol $C_2H_4 \mu g^{-1}$ protein h^{-1}) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.00^{o}	0.00^{o}	0.00^{o}	0.00^{o}	0.00
7	1.737^{e}	1.423^{f}	1.233^{g}	0.687^{j}	1.270
14	2.350^{c}	1.927^{d}	3.840^{a}	2.700^{b}	2.704
21	0.943^{i}	0.760^{j}	1.123^{h}	0.690^{j}	0.879
28	0.267^{kl}	0.190^{lm}	0.313^{k}	0.247^{klm}	0.254
35	0.217^{klm}	0.203^{lm}	0.267^{kl}	0.173^{lm}	0.215
42	0.077^{no}	0.023^{o}	0.157^{mn}	0.037^{o}	0.073
49	0.050^{o}	0.047^{o}	0.037^{o}	0.020^{o}	0.038
Mean	0.705	0.572	0.871	0.569	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.010		0.028	
Days (D)		0.014		0.039	
S × D		0.029		0.080	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

followed by *Tolypothrix tenuis* and *Nostoc muscorum* at 14th day of incubation (Table 8).

4.1.6.3 Glutamine synthetase (GS) activity (EC 6.3.1.2., μmol γ-glutamyl hydroxamate mg^{-1} protein 30 $min.^{-1}$)

Mean GS activity calculated during incubation time showed that the highest GS activity was observed at 14th day of incubation followed by a gradual decline. Enzyme activity ranged between 82.60 μmol γ-glutamyl hydroxamate mg^{-1} protein 30 $min.^{-1}$ for *Nostoc muscorum* to 24.66 μmol γ-glutamyl hydroxamate mg^{-1} protein 30 $min.^{-1}$ for *Aulosira fertilissima*. The GS activity of other two biofertilizer strains namely *Tolypothrix tenuis* and *Anabaena variabilis* was 46.36 and 41.75 μmol γ-glutamyl hydroxamate mg^{-1} protein 30 $min.^{-1}$ respectively. DMRT analysis indicated that *Nostoc muscorum* exhibited highest glutamine synthetase activity at 14th day of incubation followed by the enzyme activity observed at 7th day of incubation, which was similar to the enzyme activity shown by *Tolypothrix tenuis* at 14th day of incubation. At similar times of incubation during exponential growth, *Anabaena variabilis* recorded the placement at third group (Table 9).

4.1.6.4 Ammonia excretion (mmol NH^+_4 mL^{-1})

Study on the ammonia excretion by the biofertilizer strains is an important parameter to understand the fixed nitrogen releasing potential. Effect of incubation time on this parameter was significant with the peak release of ammonia observed at 14th day of growth.

Table 8. Nitrate reductase (NR) activity (μmol NO^{-}_{2} μg^{-1} protein) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.89^{h}	1.00^{h}	0.78^{h}	0.79^{h}	0.87
7	69.82^{de}	91.49^{d}	80.35^{de}	70.84^{de}	78.13
14	78.11^{de}	210.2^{a}	129.4^{c}	178.3^{b}	149.0
21	37.45^{fg}	69.47^{de}	65.82^{de}	55.51^{ef}	57.06
28	32.60^{fg}	28.49^{fg}	30.47^{fg}	29.32^{fg}	30.22
35	29.37^{fg}	25.07^{gh}	25.39^{gh}	23.99^{gh}	25.95
Mean	41.37	70.96	55.36	59.79	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		3.40		9.42	
Days (D)		4.16		1.15	
S × D		8.33		23.07	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

Table 9. Glutamine synthetase (GS) activity (μmol γ-glutamyl hydroxamate mg^{-1} protein 30 $min.^{-1}$) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.17^{i}	0.12^{i}	0.13^{i}	0.08^{i}	0.13
7	82.93^{d}	63.58^{e}	137.13^{b}	60.04^{e}	85.92
14	105.23^{c}	62.43^{e}	255.01^{a}	128.92^{b}	137.99
21	34.98^{fg}	7.19^{hi}	64.30^{e}	45.80^{ef}	38.07
28	19.38^{ghi}	7.91^{hi}	24.02^{gh}	27.36^{fgh}	19.67
35	7.81^{hi}	6.72^{hi}	15.05^{ghi}	15.99^{ghi}	11.39
Mean	41.75	24.66	82.60	46.36	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		2.565		7.105	
Days (D)		3.142		8.703	
S × D		6.283		17.404	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

There was a significant difference amongst the strains examined with respect to this attribute. *Anabaena variabilis* showed highest extracellular ammonia release followed by *Tolypothrix tenuis*. DMRT analysis revealed that the extracellular ammonia release was highest by *Anabaena variabilis* followed by *Tolypothrix tenuis* at 21st day of incubation followed by *Anabaena variabilis* at 14th day of incubation (Table 10).

4.2 Crop, variety and growth conditions

Studies were undertaken in relation to carbon and nitrogen contribution using four biofertilizer strains namely *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Tolypothrix tenuis* alone or in combination with three levels of nitrogenous fertilizer in comparison to control (uninoculated, -N and +N treatments) under pot culture conditions in rice crop variety Basmati-1. Experiments were conducted at National Phytotron Facility and NCCUBGA (during *kharif*, 2002), IARI, New Delhi (Plates 3a & b).

4.3 Experimental details

Pot culture experiments were conducted under controlled Phytotron conditions and under natural conditions (during *kharif* season, 2002) to examine the role of blue green algal inoculation on carbon and nitrogen levels in soil and rice crop under different levels of nitrogenous fertilizers in comparison to control uninoculated condition (Plate 4a&b). Under Phytotron conditions, rice crop was raised in peat which was analysed for only algal population under different treatment combinations. The influence

Table 10. Ammonia excretion (mmol NH^+_4 mL^{-1}) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.001^{g}	0.001^{g}	0.002^{g}	0.001^{g}	0.001
7	0.253^{fg}	0.252^{fg}	0.252^{fg}	0.249^{fg}	0.251
14	2.224^{c}	0.848^{ef}	1.638^{cd}	1.784^{cd}	11.624
21	5.068^{a}	1.501^{d}	0.826^{ef}	2.797^{b}	2.548
28	1.526^{d}	1.840^{cd}	1.299^{de}	1.690^{cd}	1.589
35	1.385^{de}	0.419^{fg}	0.363^{fg}	1.308^{de}	0.869
Mean	1.743	0.810	0.730	1.305	
		SE (m)±		**CD (P=0.05)**	
trains (S)		0.079		0.219	
Day **(D)**		0.097		0.269	
x D		0.193		0.535	

denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are significant.

Table 10. Ammonia excretion (mmol NH^+_4 mL^{-1}) in blue green algal biofertilizer strains at different days of incubation

Days	*Anabaena variabilis*	*Aulosira fertilissima*	*Nostoc muscorum*	*Tolypothrix tenuis*	Mean
0	0.001^{g}	0.001^{g}	0.002^{g}	0.001^{g}	0.001
7	0.253^{fg}	0.252^{fg}	0.252^{fg}	0.249^{fg}	0.251
14	2.224^{c}	0.848^{ef}	1.638^{cd}	1.784^{cd}	11.624
21	5.068^{a}	1.501^{d}	0.826^{ef}	2.797^{b}	2.548
28	1.526^{d}	1.840^{cd}	1.299^{de}	1.690^{cd}	1.589
35	1.385^{de}	0.419^{fg}	0.363^{fg}	1.308^{de}	0.869
Mean	1.743	0.810	0.730	1.305	
		SE (m)±		**CD (P=0.05)**	
Strains (S)		0.079		0.219	
Days (D)		0.097		0.269	
S × D		0.193		0.535	

Superscript denotes DMRT grouping; 'a' signifies the highest value and values denoted by same superscript are non significant.

Plate 3. Experimental setup for rice crop

a) Under controlled conditions in Phytotron Facility

b) Under natural conditions


a


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Plate 4. Pot culture experiment for rice crop

a) Control, uninoculated conditions

b) Inoculated with blue green algal biofertilizer strain


a
b

of different treatment combinations was studied on certain biological and chemical properties of soil during midcrop and at harvest time during natural conditions (Material and methods, 3.3).

4.4 Analysis

4.4.1 Peat

Algal abundance was analyzed in peat during midcrop and at harvest stages. Details regarding the peat used are given in material and methods (3.2). Application of nitrogenous fertilizer enhanced algal population during midcrop as well as during harvest stage. Under control (uninoculated) conditions, no algal population were detected. However, with the application of *Anabaena variabilis*, the algal population was highest throughout the crop growth. DMRT grouping for mean algal population during midcrop as well as harvest stage showed the highest population under the treatment, N_2S_1 *i.e.*, *Anabaena variabilis* inoculation under 100% N (Table 11).

4.4.2 Soil

4.4.2.1 Microbial flora

a) Algal population (CFU $\times 10^3 g^{-1}$)

i) Midcrop stage

There was a significant effect of application of increasing levels of nitrogenous fertilizers on algal population. During midcrop, the soil algal population was highest in *Anabaena variabilis* treated pots which was *at par* with *Tolypothrix tenuis* treated pots. The lowest population was observed under uninoculated control pots. DMRT

Table 11. Influence of blue green algal inoculation on algal population (CFU × 10^2 g^{-1}) in peat at different levels of Nitrogenous fertilizer under Phytotron conditions

N levels / Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	ND	ND	ND	ND	ND	ND	ND	ND
S_1	3.7	6.3	7.3	5.8	5.0	7.7	8.3	7.0
S_2	2.0	4.0	4.3	3.4	2.7	6.7	5.7	5.0
S_3	3.0	5.3	5.3	4.5	3.3	5.0	6.7	5.0
S_4	2.7	4.0	5.0	3.9	3.0	4.3	5.0	4.1
Mean	2.9	4.9	5.5		3.5	5.9	6.4	
	SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)		
N level (N)	0.44	1.19			0.38	1.03		
Strain (S)	0.50	1.35			0.44	1.19		
N × S	0.87	2.35			0.76	2.05		

ND: Not Detected

DMRT Grouping for mean performance of algal population under Phytotron conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_1S_1	AB	N_1S_1	AB
N_1S_3	ABC	N_2S_3	ABC
N_2S_3	ABC	N_1S_2	ABC
N_2S_4	ABC	N_2S_2	BCD
N_2S_2	BCD	N_0S_1	CDE
N_1S_4	BCD	N_1S_3	CDE
N_1S_2	BCD	N_2S_4	CDE
N_0S_1	BCD	N_1S_4	CDE
N_0S_3	CD	N_0S_3	DE
N_0S_4	CD	N_0S_4	E
N_0S_2	D	N_0S_2	E

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 and N_2 represents 50% and 100% N levels as in Hoagland's medium
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

analysis carried out for nitrogen levels x strains interaction studies showed that inoculation with *Tolypothrix tenuis* under 120 kg N ha^{-1} exhibited highest colony forming units followed by inoculation with *Anabaena variabilis* (Table 12.1).

ii) Harvest stage

The algal population was more under N_0 (no nitrogen) in comparison to the pots given half and recommended rates of nitrogenous fertilizer (*i.e.*, 60 kg N ha^{-1} and 120 kg N ha^{-1} respectively). Inoculation of soil with *Nostoc muscorum* and *Tolypothrix tenuis* resulted in highest CFU values and the lowest was observed under the (uninoculated) control pots. The interaction studies carried out during harvest indicated an inhibitory influence of N on algal population. The treatment involving inoculation of *Anabaena variabilis* at zero N level showed highest CFU which was *at par* with the values observed under *Nostoc muscorum* and *Tolypothrix tenuis* treatment. The lowest algal population was observed at 60 kg N ha^{-1} and at 120 kg N ha^{-1} under uninoculated conditions (Table 12.1).

b) Bacterial population (CFU $\times 10^6$ g^{-1})

i) Midcrop stage

Mean soil bacterial population increased with increase in nitrogenous fertilizer, and the influence of different blue green algal strains on soil bacterial population indicated lowest CFU under S_0, *i.e.*, uninoculated control. Highest bacterial population was recorded under *Anabaena variabilis* treatment. DMRT analysis

Table 12 Soil microbial flora

12.1 Influence of blue green algal inoculation on soil algal population (CFU × 10^3 g^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	8.73	10.33	15.00	11.36	4.80	3.13	3.47	3.80
S_1	10.13	14.67	21.67	15.49	6.17	4.20	4.33	4.90
S_2	7.33	13.00	19.00	13.11	5.50	4.10	4.30	4.63
S_3	7.67	14.00	20.00	13.89	6.07	4.77	4.20	5.01
S_4	7.33	13.33	25.00	15.22	5.97	4.77	4.50	5.08
Mean	8.24	13.07	20.13		5.70	4.19	4.16	
	SE (m)±		CD (P=0.05)		SE (m)±		CD (P=0.05)	
N level (N)	0.283		0.784		0.085		0.024	
Strain (S)	0.366		1.015		0.110		0.304	
N × S	0.633		1.755		0.191		0.053	

DMRT Grouping for mean performance of soil algal population

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_4	A	N_0S_1	A
N_2S_1	B	N_0S_3	AB
N_2S_3	BC	N_0S_4	AB
N_2S_2	C	N_0S_2	B
N_2S_0	D	N_0S_0	C
N_1S_1	D	N_1S_3	C
N_1S_3	D	N_1S_4	C
N_1S_4	D	N_2S_4	CD
N_1S_2	D	N_2S_1	CD
N_1S_0	E	N_2S_2	CD
N_0S_1	E	N_1S_1	CD
N_0S_0	EF	N_2S_3	CD
N_0S_3	F	N_1S_2	D
N_0S_2	F	N_2S_0	E
N_0S_4	F	N_1S_0	E

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
Tolypothrix tenuis

Table 12 Soil microbial flora

12.1 Influence of blue green algal inoculation on soil algal population (CFU × 10^3 g^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	8.73	10.33	15.00	11.36	4.80	3.13	3.47	3.80
S_1	10.13	14.67	21.67	15.49	6.17	4.20	4.33	4.90
S_2	7.33	13.00	19.00	13.11	5.50	4.10	4.30	4.63
S_3	7.67	14.00	20.00	13.89	6.07	4.77	4.20	5.01
S_4	7.33	13.33	25.00	15.22	5.97	4.77	4.50	5.08
Mean	8.24	13.07	20.13		5.70	4.19	4.16	
	SE (m)±		CD (P=0.05)		SE (m)±		CD (P=0.05)	
N level (N)	0.283		0.784		0.085		0.024	
Strain (S)	0.366		1.015		0.110		0.304	
N × S	0.633		1.755		0.191		0.053	

DMRT Grouping for mean performance of soil algal population

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_4	A	N_0S_1	A
N_2S_1	B	N_0S_3	AB
N_2S_3	BC	N_0S_4	AB
N_2S_2	C	N_0S_2	B
N_2S_0	D	N_0S_0	C
N_1S_1	D	N_1S_3	C
N_1S_3	D	N_1S_4	C
N_1S_4	D	N_2S_4	CD
N_1S_2	D	N_2S_1	CD
N_1S_0	E	N_2S_2	CD
N_0S_1	E	N_1S_1	CD
N_0S_0	EF	N_2S_3	CD
N_0S_3	F	N_1S_2	D
N_0S_2	F	N_2S_0	E
N_0S_4	F	N_1S_0	E

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

indicated that the highest bacterial population was observed under 120 kg N ha^{-1} in presence of *Anabaena variabilis, Tolypothrix tenuis, Nostoc muscorum* and *Aulosira fertilissima*. The lowest soil bacterial population was observed in uninoculated control pots, under *Aulosira fertilissima* and *Tolypothrix tenuis* inoculated pots where no nitrogenous fertilizer was given (Table 12.2).

ii) Harvest stage

Even at harvest stage, increase in the level of nitrogenous fertilizer enhanced the soil bacterial population and the blue green algal application increased the soil bacterial population which was significantly higher than that of uninoculated control conditions with *Anabaena variabilis* exhibiting the highest influence. The effect of treatment N_2S_1 (120 kg N ha^{-1}, *Anabaena variabilis* inoculation) and N_2S_3 (120 kg N ha^{-1}, *Nostoc muscorum* inoculation) on bacterial population was the highest and uninoculated pots under zero level of nitrogenous fertilizer exhibited lowest soil bacterial population (Table 12.2).

C) Fungal population (CFU $\times 10^2$ g^{-1})

i) Midcrop stage

Soil fungal population exhibited a marked enhancement with the application of nitrogenous fertilizers. Comparison of blue green algal strains on fungal population showed that the inoculation with *Tolypothrix tenuis* produced maximum soil fungal population and with *Anabaena variabilis produced* lower fungal population. Under uninoculated control conditions, the fungal population was lowest. Interaction studies indicated that the highest fungal population was

12.2 Influence of blue green algal inoculation on soil bacterial populations (CFU × 10^6 g^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	35.67	40.00	51.33	42.33	36.67	41.33	50.33	42.78
S_1	38.67	49.67	58.00	48.78	44.00	44.67	53.33	47.33
S_2	35.00	50.67	55.67	47.11	42.00	44.33	52.00	46.11
S_3	37.00	48.00	56.00	47.00	41.00	45.67	52.67	46.44
S_4	36.33	49.33	56.33	47.32	41.33	41.67	51.67	44.89
Mean	36.53	47.53	55.47		41.00	43.53	52.00	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.33	0.92			0.29	0.80	
Strain (S)		0.43	1.19			0.37	1.03	
N × S		0.73	2.01			0.64	1.79	

DMRT Grouping for mean performance of soil bacterial population

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_4	A	N_2S_3	A
N_2S_3	A	N_2S_2	AB
N_2S_2	A	N_2S_4	AB
N_1S_2	B	N_2S_0	B
N_2S_0	B	N_1S_3	C
N_1S_1	BC	N_1S_1	C
N_1S_4	BC	N_1S_2	C
N_1S_3	C	N_0S_1	C
N_1S_0	D	N_0S_2	D
N_0S_1	DE	N_1S_4	D
N_0S_3	EF	N_0S_4	D
N_0S_4	F	N_1S_0	D
N_0S_0	F	N_0S_3	D
N_0S_2	F	N_0S_0	E

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

observed at 120 kg N ha^{-1} under *Tolypothrix tenuis* inoculation followed by inoculation with *Anabaena variabilis* and *Aulosira fertilissima*. The lowest fungal population was observed under control uninoculated treatment at zero level of nitrogenous fertilizer (Table 12.3).

ii) Harvest stage

Increase in the levels of nitrogenous fertilizer enhanced soil fungal population. Inoculation with the biofertilizer strains improved the soil fungal population in comparison to that of uninoculated control with the highest being observed with *Nostoc muscorum* inoculation. DMRT analysis indicated that nitrogen application @ 120 kg N ha^{-1} in the presence of *Aulosira fertilissima* and *Nostoc muscorum* showed highest fungal population followed by *Tolypothrix tenuis* and *Anabaena variabilis* respectively (Table 12.3).

d) Actinomycetes (CFU $\times 10^4$ g^{-1})

i) Midcrop stage

Soil actinomycetes population was affected and improved with the application of nitrogenous fertilizer ranging from 2.13 (CFU x 10^4 g^{-1} at N_0) to 7.33 (CFU x 10^4 g^{-1} at 120 kg N ha^{-1}). The actinomycetes population was improved with application of blue green algal biofertilizer strains in comparison to that of control and the best effect was observed with the application of *Aulosira fertilissima*. The highest actinomycetes population was observed under *Aulosira fertilissima* at 120 kg N ha^{-1} and the lowest level was

12.3 Influence of blue green algal inoculation on soil fungal population (CFU × 10^2 g^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	16.67	21.00	25.00	20.89	12.67	18.00	24.33	18.33
S_1	23.67	26.67	30.00	26.78	18.00	21.00	26.00	21.67
S_2	21.33	25.00	30.00	25.44	17.00	19.33	28.00	21.44
S_3	23.00	26.00	28.67	25.89	17.67	22.00	28.33	22.67
S_4	23.00	25.67	39.33	29.33	17.00	23.00	27.33	22.44
Mean	21.53	24.87	30.60		16.47	20.67	26.80	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.32	0.89			0.24	0.67	
Strain (S)		0.42	1.15			0.31	0.86	
N × S		0.72	2.00			0.54	1.49	

DMRT Grouping for mean performance of soil fungal population

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_4	A	N_2S_3	A
N_2S_1	B	N_2S_2	A
N_2S_2	B	N_2S_4	AB
N_2S_3	BC	N_2S_1	B
N_1S_1	CD	N_2S_0	C
N_1S_3	D	N_1S_4	CD
N_1S_4	DE	N_1S_3	DE
N_1S_2	DEF	N_1S_1	E
N_2S_0	DEF	N_1S_2	F
N_0S_1	EF	N_0S_1	FG
N_0S_3	FG	N_1S_0	FG
N_0S_4	FG	N_0S_3	FG
N_1S_0	G	N_0S_2	G
N_0S_2	G	N_0S_4	G
N_0S_0	H	N_0S_0	H

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

observed under uninoculated and *Tolypothrix tenuis* inoculated pots in absence of nitrogenous fertilizers (Table 12.4).

ii) Harvest stage

During harvest, there was a marked enhancement in soil actinomycetes with application of nitrogenous fertilizer. *Anabaena variabilis* had the most significant influence on the soil actinomycetes population than the other blue green algal strains examined. DMRT studies indicated that *Tolypothrix tenuis* inoculation at 120 kg N ha^{-1} revealed highest population of actinomycetes which was at par with *Anabaena variabilis* at similar N level inoculation and the lowest soil actinomycetes population was observed under the treatment where no nitrogen was given in the uninoculated pots (Table 12.4).

4.4.2.2 pH and EC (dS m^{-1})

i) Midcrop stage

Inoculation with blue green algal biofertilizer strains had no influence on soil pH under different levels of nitrogenous fertilizer and this parameter remained more or less constant and varied between 7.35 to7.64 in the presence of different blue green algal strains (Table 13, Fig.1). Treatment combination N_1S_2 (60 kg N ha^{-1} and *Aulosira fertilissima* inoculation) was grouped at highest level based upon DMRT grouping. Increase in N application marginally enhanced soil EC. With the application biofertilizer strains, there was not much influence on this parameter. This attribute was highest under *Anabaena variabilis*

12.4 Influence of blue green algal inoculation on soil actinomycetes population (CFU × 10^4 g^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	1.33	2.33	3.33	2.33	1.67	3.67	4.67	3.33
S_1	3.33	6.67	8.33	6.11	4.67	6.67	7.67	6.33
S_2	3.00	6.67	10.00	6.56	3.67	5.00	6.33	5.00
S_3	1.67	7.00	9.67	6.11	4.33	5.67	7.33	5.78
S_4	1.33	3.67	5.33	3.44	3.67	4.67	8.00	5.44
Mean	2.13	5.27	7.33		3.60	5.13	6.80	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.23	0.64			0.17	0.47	
Strain (S)		0.30	0.83			0.22	0.60	
N × S		0.52	0.43			0.38	1.04	

DMRT Grouping for mean performance of soil actinomycetes population

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_2	A	N_2S_4	A
N_2S_3	AB	N_2S_1	AB
N_2S_1	BC	N_2S_3	ABC
N_1S_3	CD	N_1S_1	BCD
N_1S_1	DE	N_2S_2	CD
N_1S_2	DE	N_1S_3	DE
N_2S_4	E	N_1S_2	EF
N_1S_4	F	N_0S_1	EFG
N_0S_1	F	N_1S_4	EFG
N_2S_0	F	N_2S_0	EFG
N_0S_2	FG	N_0S_3	FG
N_1S_0	FGH	N_0S_2	G
N_0S_3	GH	N_0S_4	G
N_0S_0	H	N_1S_0	G
N_0S_4	H	N_0S_0	H

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

Table 13. Influence of blue green algal inoculation on soil pH under different levels of nitrogenous fertilizer

N levels/	Midcrop stage				Harvest stage			
Strains	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	7.31	7.39	7.34	7.35	7.59	8.10	8.13	7.94
S_1	7.62	7.65	7.65	7.64	8.27	8.50	8.41	8.39
S_2	7.53	7.67	7.50	7.56	8.33	8.37	8.31	8.34
S_3	7.46	7.57	7.52	7.52	8.27	8.53	8.34	8.38
S_4	7.38	7.40	7.52	7.43	8.27	8.23	8.30	8.27
Mean	7.46	7.54	7.51		8.15	8.35	8.30	

		SE (m)±	CD (P=0.05)
N level (N)	NS	0.018	0.050
Strain (S)	NS	0.024	0.067
N × S	NS	0.041	0.114

DMRT Grouping for mean performance of soil pH

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_1S_2	A	N_1S_3	A
N_1S_1	AB	N_1S_1	A
N_2S_1	AB	N_2S_1	AB
N_0S_1	AB	N_1S_2	BC
N_1S_3	BC	N_2S_3	BC
N_0S_2	CD	N_0S_2	BC
N_2S_3	CD	N_2S_2	BC
N_2S_4	CD	N_2S_4	BC
N_2S_2	CD	N_0S_1	C
N_0S_3	DE	N_0S_3	C
N_1S_4	EF	N_0S_4	C
N_1S_0	EFG	N_1S_4	CD
N_0S_4	EFG	N_2S_0	DE
N_2S_0	FG	N_1S_0	E
N_0S_0	G	N_0S_0	F

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

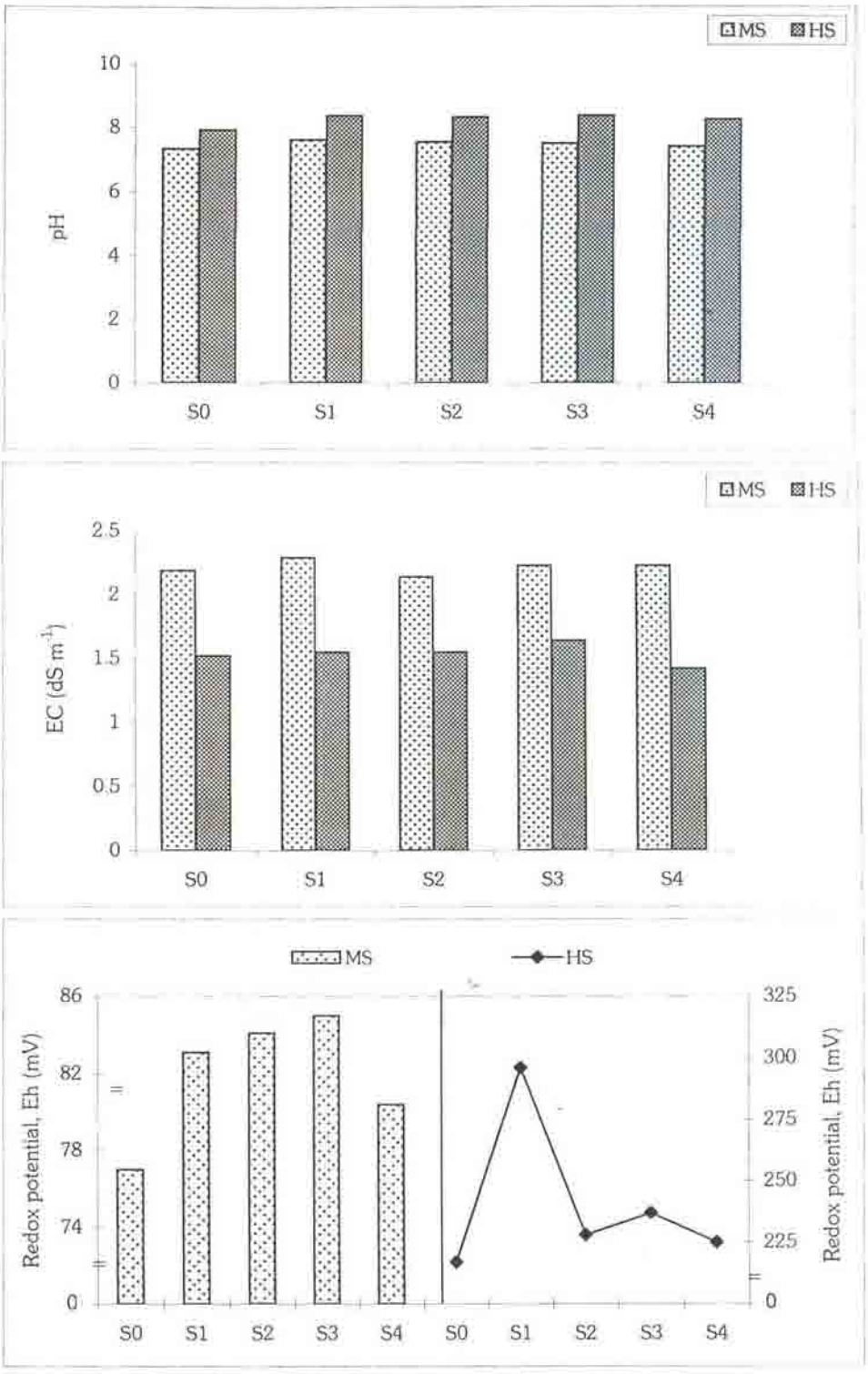


Fig.1 Influence of blue green algal inoculation on specific soil parameters (pH, EC and redox potential) under natural conditions

MS- Midcrop stage; HS- Harvest stage

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

inoculation and under *Nostoc muscorum* inoculation at 120 kg N ha^{-1} based upon DMRT analysis (Table 14, Fig. 1).

ii) Harvest stage

There was a slight enhancement in mean soil pH with the application of blue green algal strains in comparison to control conditions. The different levels of nitrogenous fertilizer did not have an appreciable influence on the soil pH level and the highest pH was recorded under the treatment N_1S_3 and N_1S_1 *i.e.* under *Anabaena variabilis* and *Nostoc muscorum* inoculation at 60 kg N ha^{-1} (Table 13, Fig.1). During harvest, there was no significant influence of biofertilizer strains and nitrogenous fertilizer application on EC. DMRT analysis indicated that *Nostoc muscorum* inoculation with 120 kg N ha^{-1} was grouped as highest treatment combination (Table 14, Fig.1).

4.4.2.3 Redox potential (mV)

i) Midcrop stage

Redox potential was more at N_2 (120 kg ha^{-1}) than that at N_1 (60 kg N ha^{-1}) during midcrop stage. Highest redox potential was observed with *Nostoc muscorum* inoculation and lowest was recorded under uninoculated control. Redox potential was highest and similar with inoculation of *Nostoc muscorum, Tolypothrix tenuis* and *Aulosira fertilissima* with nitrogen application at the rate of 120 kg N ha^{-1} (Table 15, Fig.1).

Table 14. Influence of blue green algal inoculation on soil EC (dS m^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	1.69	2.39	2.49	2.19	1.33	1.60	1.63	1.52
S_1	1.78	2.49	2.51	2.29	1.55	1.61	1.47	1.55
S_2	1.62	2.33	2.45	2.14	1.52	1.63	1.53	1.55
S_3	1.59	2.48	2.51	2.23	1.52	1.60	1.80	1.64
S_4	1.72	2.42	2.55	2.23	1.55	1.45	1.25	1.42
Mean	1.68	2.42	2.54		1.50	1.58	1.54	
N level (N)		NS				NS		
Strain (S)		NS				NS		
N × S		NS				NS		

DMRT Grouping for mean performance of soil EC

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_3	A
N_2S_3	A	N_2S_4	B
N_2S_4	AB	N_1S_2	AB
N_2S_0	AB	N_2S_0	AB
N_1S_3	ABC	N_1S_1	AB
N_1S_1	ABC	N_1S_0	AB
N_2S_2	ABC	N_1S_3	AB
N_1S_4	ABCD	N_2S_2	AB
N_1S_0	ABCD	N_0S_1	AB
N_1S_2	ABCD	N_0S_4	AB
N_0S_1	ABCD	N_0S_2	AB
N_0S_4	BCD	N_0S_3	AB
N_0S_0	BCD	N_2S_1	AB
N_0S_2	CD	N_1S_4	AB
N_0S_3	D	N_0S_0	AB

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

Table 15. Influence of blue green algal inoculation on soil redox potential (mV) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	64.0	39.3	127.7	77.0	133	190	329	217
S_1	71.7	78.3	99.3	83.1	184	317	386	296
S_2	69.0	55.0	128.3	84.1	132	222	330	228
S_3	67.7	54.3	133.0	85.0	136	204	371	237
S_4	61.0	51.0	129.3	80.4	134	201	341	225
Mean	66.7	55.6	123.5		144	227	351	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		1.86	5.0			4.22	11.4	
Strain (S)		2.40	6.5			5.45	14.7	
N × S		4.16	11.2			9.44	25.5	

DMRT Grouping for mean performance of soil redox potential

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_3	A	N_2S_1	A
N_2S_4	A	N_2S_3	A
N_2S_2	A	N_2S_4	B
N_2S_0	A	N_2S_2	B
N_2S_1	B	N_2S_0	B
N_1S_1	C	N_1S_1	B
N_0S_1	CD	N_1S_2	C
N_0S_2	CD	N_1S_3	CD
N_0S_3	CDE	N_1S_4	CD
N_0S_4	DEF	N_1S_0	D
N_0S_0	DEF	N_0S_1	D
N_1S_2	EF	N_0S_3	E
N_1S_3	F	N_0S_4	E
N_1S_4	FG	N_0S_0	E
N_1S_0	G	N_0S_2	E

MRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
MRT grouping in descending order with highest denoted by alphabet A
Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
Tolypothrix tenuis

ii) Harvest stage

At the time of harvest of rice crop, the redox potential enhanced with increase in nitrogenous fertilizer application. Out of different biofertilizer strains examined, *Anabaena variabilis* inoculation recorded maximum soil redox potential. In presence of nitrogenous fertilizer @ 120 kg N ha^{-1}, the redox potential was highest with the application of *Anabaena variabilis* and *Nostoc muscorum* followed by the levels observed with the application of *Tolypothrix tenuis*, *Aulosira fertilissima* and uninoculated control (Table 15, Fig.1).

4.4.2.4 Chlorophyll ($\mu g\ g^{-1}$)

i) Midcrop stage

The soil chlorophyll content increased with an enhancement in the rate of nitrogenous fertilizer application. This content increased from 44.45 $\mu g\ g^{-1}$ under N_0 (No nitrogen) to 104.29 $\mu g\ g^{-1}$ under N_2 (120 kg N ha^{-1}). There was a significant influence of biofertilizer strains on the soil chlorophyll. The highest soil chlorophyll content was exhibited by *Tolypothrix tenuis* inoculation and the lowest content was observed in uninoculated control pots. The lowest soil chlorophyll content observed was less than half in comparison to the soil chlorophyll content observed under *Tolypothrix tenuis* inoculation. DMRT analysis indicated the highest soil chlorophyll content under the treatment N_2 S_4 (120 kg N ha^{-1} + inoculation with *Tolypothrix tenuis*) which was more than 85% over the –N treatment where no biofertilizer inoculum was provided. The

highest chlorophyll content under N_2S_4 treatment was followed by the chlorophyll content under the treatment N_2S_1 with *Anabaena variabilis* inoculation (Table 16, Fig.2).

ii) Harvest stage

When blue green algal biofertilizer strains were compared, during harvest, analysis carried out indicated that inoculation with *Anabaena variabilis* resulted in highest soil chlorophyll while that with *Aulosira fertilissima* exhibited lowest soil chlorophyll content which was higher than the soil chlorophyll content observed under control conditions. Increase in nitrogen levels form 0 to 60 kg N ha^{-1} and further to 120 kg N ha^{-1} enhanced soil chlorophyll content significantly. As per DMRT analysis, the treatment combination namely *Anabaena variabilis* inoculation and 120 kg N ha^{-1} was the best with respect to soil chlorophyll analysed followed by treatment with *Tolypothrix tenuis* under similar level of N fertilizer (Table 16, Fig.2).

4.4.2.5 Dehydrogenase activity (μl H_2 g^{-1} day^{-1})

i) Midcrop stage

Soil dehydrogenase activity was influenced significantly with the increase in rates of nitrogenous fertilizer. The activity was more than two fold under the treatment N_2 i.e., 120 kg N ha^{-1} in comparison to the activity observed in N_0 *i.e.,* without nitrogen. There was a significant effect of different biofertilizer inoculation on this attribute and highest activity was observed under inoculation with *Tolypothrix tenuis*. Inoculation of soil with *Aulosira fertilissima*

Table 16. Influence of blue green algal inoculation on soil chlorophyll content ($\mu g\ g^{-1}$) under different levels of nitrogenous fertilizer

N levels/ Strain	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	23.34	30.92	48.97	34.41	6.87	12.71	39.20	19.2
S_1	64.90	83.48	114.71	87.70	21.67	48.64	65.41	45.3
S_2	43.36	76.01	87.89	69.09	11.89	31.25	44.38	29.
S_3	51.45	58.12	107.27	72.28	19.13	41.95	54.25	38.4
S_4	39.21	65.41	162.59	89.07	8.06	26.03	61.39	31.5
Mean	44.45	62.78	104.29		13.52	32.12	52.92	

	SE (m)±	CD (P=0.05)	SE (m)±	CD (P=0.05)
N level (N)	0.93	2.58	0.58	1.62
Strain (S)	1.20	3.33	0.75	2.08
N × S	2.08	5.77	1.30	3.61

DMRT Grouping for mean performance of soil chlorophyll content

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_4	A	N_2S_1	A
N_2S_1	B	N_2S_4	B
N_2S_3	C	N_2S_3	C
N_2S_2	D	N_1S_1	D
N_1S_1	D	N_2S_2	E
N_1S_2	E	N_1S_3	EF
N_1S_4	F	N_2S_0	F
N_0S_1	F	N_1S_2	H
N_1S_3	G	N_1S_4	H
N_0S_3	H	N_0S_1	I
N_2S_0	HI	N_0S_3	I
N_0S_2	IJ	N_1S_0	J
N_0S_4	J	N_0S_2	J
N_1S_0	K	N_0S_4	K
N_0S_0	L	N_0S_0	K

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

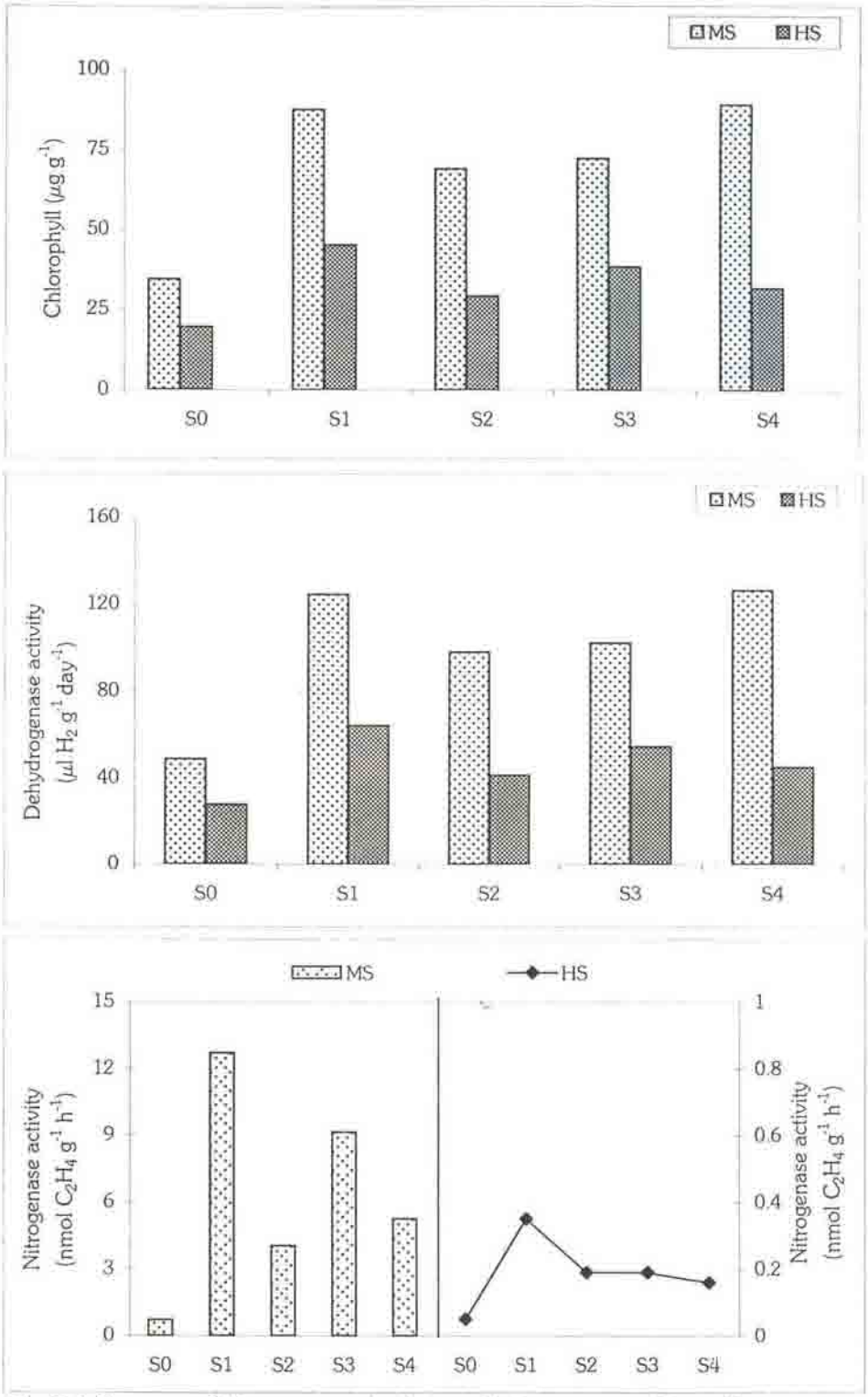


Fig.2 Influence of blue green algal inoculation on specific soil parameters (chlorophyll, dehydrogenase and nitrogenase activity) under natural conditions

MS- Midcrop stage; HS- Harvest stage

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

had minimum impact on soil dehydrogenase activity in comparison to other biofertilizer strains examined. The dehydrogenase activity was lowest under control pots *i.e.,* without inoculation. DMRT analysis indicated that the highest dehydrogenase activity was observed under the inoculation with *Tolypothrix tenuis* in presence of 120 kg N ha^{-1}. At similar rates of nitrogenous fertilizer, *Anabaena variabilis* inoculation resulted in slighter lower activity. The lowest dehydrogenase activity, under the treatment where the pots did not receive any nitrogenous fertilizer and biofertilizer inoculation was seven times less in comparison to the highest dehydrogenase activity. Minimal activity observed under the control pots could be attributed to the indigenous microbial population established actively during the midcrop phase (Table 17, Fig.2).

ii) Harvest stage

There was a significant effect of increasing levels of nitrogenous fertilizer on soil dehydrogenase activity with the highest at N_2, *i.e.,* 120 kg N ha^{-1} (75.15 μl H_2 g^{-1} day^{-1}) and lowest at N_0 i.e., without N (19.20 μl H_2 g^{-1} day^{-1}). There was a marked influence of biofertilizer strains on dehydrogenase activity in comparison to control and the inoculation with *Anabaena variabilis* resulted in highest dehydrogenase activity. During harvest, the highest dehydrogenase activity was observed with *Anabaena variabilis* inoculation at 120 kg N ha^{-1} followed by *Tolypothrix tenuis* inoculation at similar level of nitrogenous fertilizer (Table 17, Fig.2).

Table 17. Influence of blue green algal inoculation on soil dehydrogenase activity (μl H_2 g^{-1} day^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	33.14	43.90	69.53	48.86	9.75	18.04	55.66	27.82
S_1	92.16	118.54	162.89	124.53	30.77	69.07	92.88	64.24
S_2	61.58	107.94	124.80	98.11	16.88	44.38	63.01	41.42
S_3	73.06	82.51	152.33	102.33	27.16	59.57	77.04	54.59
S_4	55.69	92.88	230.87	126.48	11.44	36.97	87.18	45.19
Mean	63.13	89.15	148.09		19.20	45.60	75.15	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		1.32	3.66			0.83	0.23	
Strain (S)		1.71	4.73			1.07	2.96	
N × S		2.96	8.19			1.85	5.13	

DMRT Grouping for mean performance of soil dehydrogenase activity

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_4	A	N_2S_1	A
N_2S_1	B	N_2S_4	B
N_2S_3	C	N_2S_3	C
N_2S_2	D	N_1S_1	D
N_1S_1	D	N_2S_2	E
N_1S_2	E	N_1S_3	EF
N_1S_4	F	N_2S_0	F
N_0S_1	F	N_1S_2	G
N_1S_3	G	N_1S_4	H
N_0S_3	H	N_0S_1	I
N_2S_0	HI	N_0S_3	I
N_0S_2	IJ	N_1S_0	J
N_0S_4	J	N_0S_2	J
N_1S_0	K	N_0S_4	K
N_0S_0	L	N_0S_0	K

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

4.4.2.6 Nitrogenase activity (nmol C_2H_4 g^{-1} h^{-1})

i) Midcrop stage

Nitrogenase activity differed significantly under different levels of nitrogenous fertilizers. The activity was highest under 60 kg N ha^{-1} and was reduced under higher application of nitrogenous fertilizer. Inoculation of soil with *Anabaena variabilis* resulted in highest nitrogenase activity which was significantly higher than the activity observed with *Nostoc muscorum* inoculation. Inoculation with *Aulosira fertilissima* produced lowest nitrogenase activity (4.05 nmol C_2H_4 g^{-1} h^{-1}) out of the four biofertilizer strains examined. Interaction studies showed that the highest nitrogenase activity was observed with *Nostoc muscorum* inoculation at 60 kg N ha^{-1} which was *at par* with the activity observed with *Anabaena variabilis* inoculation under 120 kg N ha^{-1}. The lowest nitrogenase activity was observed in uninoculated pots under N_0 (without nitrogen), N_1 (60 kg N ha^{-1}) and under N_2 (120 kg N ha^{-1}) and also under the treatment where *Tolypothrix tenuis* was applied under N_0 (Table 18, Fig.2).

ii) Harvest stage

Increase in the level of nitrogenous fertilizer did not affect nitrogenase activity much. The highest activity was observed under *Anabaena variabilis* application and the lowest activity recorded was obviously under uninoculated control pots. During harvest, highest soil nitrogenase activity was observed at intermediate level of nitrogenous fertilizer (*i.e.* 60 kg N ha^{-1}) followed by the activity observed at 120 kg N ha^{-1} with the inoculation of *Anabaena variabilis*. This activity

Table 18. Influence of blue green algal inoculation on soil nitrogenase activity (Acetylene Reduction Assay, nmol C_2H_4 g^{-1} h^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	0.55	0.78	0.82	0.72	0.35	0.04	0.06	0.05
S_1	9.08	12.40	16.69	12.72	0.30	0.44	0.32	0.35
S_2	4.26	3.40	4.48	4.05	0.15	0.25	0.17	0.19
S_3	2.74	17.40	7.34	9.16	0.18	0.15	0.25	0.19
S_4	0.96	9.62	5.23	5.27	0.10	0.29	0.10	0.16
Mean	3.52	8.72	6.91		0.15	0.23	0.18	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.185	0.513			0.010	0.03	
Strain (S)		0.239	0.662			0.013	0.04	
N × S		0.414	1.148			0.022	0.59	

DMRT Grouping for mean performance of soil nitrogenase activity

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_1S_3	A	N_1S_1	A
N_2S_1	A	N_2S_1	B
N_1S_1	B	N_0S_1	BC
N_1S_4	C	N_1S_4	BC
N_0S_1	C	N_2S_3	C
N_2S_3	D	N_1S_2	C
N_2S_4	E	N_0S_3	D
N_2S_2	EF	N_2S_2	D
N_0S_2	EF	N_1S_3	DE
N_1S_2	FG	N_0S_2	DE
N_0S_3	G	N_0S_4	EF
N_0S_4	H	N_2S_4	EF
N_2S_0	H	N_2S_0	FG
N_1S_0	H	N_1S_0	G
N_0S_0	H	N_0S_0	G

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 50 kg N ha^{-1}; N_2 = 100 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

observed was *at par* with the activity recorded under N_0 with *Anabaena variabilis* application and under N_1 with *Tolypothrix tenuis* application (Table 18, Fig.2).

4.4.2.7 Microbial biomass carbon (mg kg^{-1})

i) Midcrop stage

Soil microbial biomass carbon increased with increase in the levels of nitrogenous fertilizers. This parameter was four times more in presence of 120 kg N ha^{-1} in comparison to the amount observed under no nitrogen conditions. *Anabaena variabilis* inoculation increased the soil microbial biomass carbon significantly and was highest in comparison to other biofertilizer strains tested. This attribute was lowest under the treatment where no biofertilizer application was given. Interaction studies on the basis of DMRT analysis between blue green algal biofertilizer strains and different levels of nitrogenous fertilizer indicated a highest microbial biomass carbon observed under the treatment N_2S_4 *i.e., Tolypothrix tenuis* inoculation under 120 kg N ha^{-1} and the influence of *Anabaena variabilis* inoculation on this parameter was next in the order. This attribute was lowest in the pots where no biofertilizer inoculation was provided under zero level of nitrogenous fertilizer. The highest soil microbial biomass C was 20 times more under S_4 N_2 (*i.e., Tolypothrix tenuis* inoculation and 120 kg N ha^{-1}) than that under control pots (Table 19, Fig.3).

Table 19. Influence of blue green algal inoculation on soil microbial biomass carbon (mg kg^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	6.49	20.94	73.45	33.63	5.02	7.97	28.02	13.67
S_1	45.10	74.63	117.70	79.15	22.13	41.00	84.96	49.36
S_2	25.37	56.90	92.04	58.10	12.69	25.37	73.16	37.07
S_3	36.28	64.31	102.07	67.55	16.81	30.38	65.78	37.66
S_4	15.05	51.62	130.98	65.88	8.56	33.92	84.07	42.18
Mean	25.66	53.68	103.25		13.04	27.73	67.20	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		1.30	3.61			0.90	2.49	
Strain (S)		1.68	4.66			1.16	3.19	
N × S		2.91	8.08			2.01	5.56	

DMRT Grouping for mean performance of soil microbial biomass carbon

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_4	A	N_2S_1	A
N_2S_1	B	N_2S_4	A
N_2S_3	C	N_2S_2	B
N_2S_2	D	N_2S_3	C
N_1S_1	E	N_1S_1	D
N_2S_0	E	N_1S_4	E
N_1S_3	F	N_1S_3	EF
N_1S_2	FG	N_2S_0	EFG
N_1S_4	GH	N_1S_2	FG
N_0S_1	H	N_0S_1	GH
N_0S_3	I	N_0S_3	HI
N_0S_2	J	N_0S_2	IJ
N_1S_0	JK	N_0S_4	JK
N_0S_4	K	N_1S_0	JK
N_0S_0	L	N_0S_0	K

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

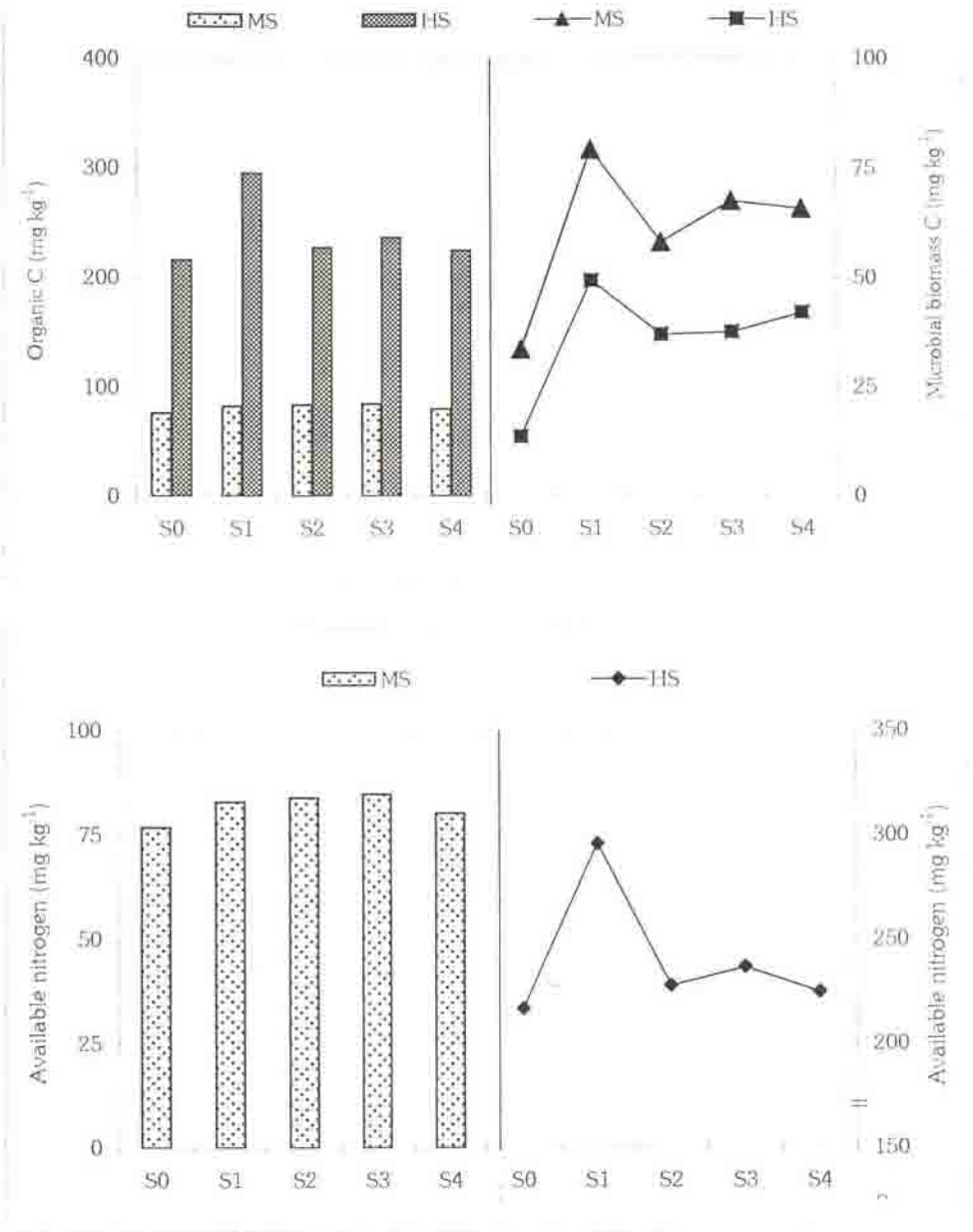


Fig.3 Influence of blue green algal inoculation on specific soil parameters (microbial biomass carbon, organic carbon and available nitrogen) under natural conditions

MS- Midcrop stage, HS- Harvest stage

S0-Without inoculation, S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

ii) Harvest stage

The increase in nitrogenous fertilizer application enhanced soil microbial biomass carbon significantly. Inoculation with *Anabaena variabilis* showed the highest enhancement while *Aulosira fertilissima* inoculation exhibited lowest soil microbial biomass carbon which was *at par* with the soil microbial biomass carbon observed under *Nostoc muscorum* inoculation. However, the samples taken from (uninoculated) control pots showed lowest soil microbial biomass carbon. Analysis of strains x nitrogenous fertilizer interaction indicated that the highest soil microbial biomass carbon was observed in soils inoculated with *Anabaena variabilis* and with *Tolypothrix tenuis* at 120 kg N ha^{-1} followed *Aulosira fertilissima* inoculation (Table 19, Fig.3).

4.4.2.8 Soil organic carbon *(%)*

i) Midcrop stage

Blue green algal inoculation had no significant influence on soil organic carbon with increased levels of nitrogenous fertilizers. However, inoculation with *Nostoc muscorum* resulted in highest organic carbon (%) when compared to uninoculated (control) pots. DMRT analysis indicated that the inoculation with *Nostoc muscorum* at 60 kg N ha^{-1} and at 120 kg N ha^{-1} resulted in higher soil organic carbon content in comparison to other blue green algal strains tested in the present study. The lowest organic carbon was observed under 120 kg N ha^{-1} in the soil sample taken from uninoculated control pots (Table 20, Fig.3).

Table 20. Influence of blue green algal inoculation on soil organic carbon (%) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	0.533	0.467	0.338	0.466	0.590	0.505	0.502	0.532
S_1	0.576	0.619	0.528	0.574	0.705	1.914	1.003	1.207
S_2	0.571	0.543	0.657	0.590	0.695	0.591	0.657	0.648
S_3	0.514	0.690	0.705	0.636	0.693	0.724	0.771	0.729
S_4	0.491	0.519	0.571	0.527	0.657	0.600	0.781	0.679
Mean	0.537	0.568	0.560		0.668	0.867	0.743	

		SE (m)±	CD (P=0.05)
N level (N)	NS	0.015	0.042
Strain (S)	NS	0.019	0.053
N × S	NS	0.033	0.092

DMRT Grouping for mean performance of soil organic carbon

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_3	A	N_1S_1	A
N_1S_3	A	N_2S_1	B
N_2S_2	AB	N_2S_4	C
N_1S_1	ABC	N_2S_3	C
N_0S_1	ABC	N_1S_3	CD
N_0S_2	ABC	N_0S_1	CD
N_2S_4	ABC	N_0S_2	CDE
N_1S_2	BC	N_0S_3	CDE
N_0S_0	BC	N_0S_4	DEF
N_2S_1	BC	N_2S_2	DEF
N_1S_4	BC	N_1S_4	EFG
N_0S_3	BC	N_0S_0	FG
N_0S_4	C	N_1S_2	FG
N_1S_0	CD	N_1S_0	G
N_2S_0	D	N_2S_0	G

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

ii) Harvest stage

With N fertilization, a slight increase in soil organic carbon content (%) was observed in comparison to that of control. Soil inoculation with *Anabaena variabilis* resulted in the highest soil organic carbon content and the content was lowest under the uninoculated conditions. Interaction studies indicated that the highest organic carbon was under the treatment N_1 S_1 (60 kg N ha^{-1}) followed by organic carbon content observed at 120 kg N ha^{-1} with *Anabaena variabilis* inoculation. Control pots without biofertilizer application in presence of 120 kg N ha^{-1} showed lowest soil organic carbon content (Table 20, Fig.3).

4.4.2.9 Available nitrogen (kg ha^{-1})

i) Midcrop stage

During midcrop, available nitrogen enhanced significantly with increase in application of nitrogenous fertilizer. Highest available nitrogen was observed with the application of *Anabaena variabilis* and the lowest was observed in control pots. DMRT analysis showed that the treatment combination of 120 kg N ha^{-1} with *Anabaena variabilis* inoculation was grouped as A and uninoculated control pots under zero level of nitrogenous fertilizer was grouped as L, lowest (Table 21, Fig.3).

ii) Harvest stage

During harvest, samples analysed showed enhanced available nitrogen content with the increase in the N application. *Anabaena variabilis* inoculation resulted in highest available nitrogen with the lowest observed under control uninoculated conditions. Under the

Table 21. Influence of blue green algal inoculation on soil available nitrogen (kg ha^{-1}) under different levels of nitrogenous fertilizer

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	160	188	210	186	153	175	215	181
S_1	181	202	247	210	180	206	241	209
S_2	175	193	220	196	169	186	223	193
S_3	180	201	233	205	175	196	231	201
S_4	167	198	215	193	169	193	224	195
Mean	173	196	225		169	191	227	
	SE (m)±		CD (P=0.05)		SE (m)±		CD (P=0.05)	
N level (N)	0.75		2.09		0.86		2.39	
Strain (S)	0.97		2.70		1.11		3.08	
N × S	1.68		4.66		1.93		5.34	

DMRT Grouping for mean performance of soil available nitrogen content of soil

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_2	C	N_2S_4	C
N_2S_4	D	N_2S_2	C
N_2S_0	E	N_2S_0	D
N_1S_1	F	N_1S_1	E
N_1S_3	F	N_1S_3	F
N_1S_4	F	N_1S_4	F
N_1S_2	G	N_1S_2	G
N_1S_0	H	N_0S_1	H
N_0S_1	I	N_0S_3	HI
N_0S_3	IJ	N_1S_0	HI
N_0S_2	J	N_0S_2	I
N_0S_4	K	N_0S_4	I
N_0S_0	L	N_0S_0	J

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

treatment combination N_2S_1 *i.e.,* 120 kg N ha^{-1} with *Anabaena variabilis* application, available nitrogen content was the highest (Table 21, Fig.3).

4.4.3 Plants

4.4.3.1 Dry matter production (g pot^{-1})

i) Midcrop stage

The increase in nitrogenous fertilizer application enhanced plant dry matter markedly in control Phytotron as well as natural conditions. There was a significant influence of blue green algal inoculation on this attribute with the highest content observed under *Anabaena variabilis* inoculation. The lowest plant dry matter production was observed under uninoculated control pots. DMRT analysis carried out indicated that the treatment combination N_2S_1 *i.e.,* 100% N with *Anabaena variabilis* inoculation was the best in influencing this parameter during midcrop stage (Tables 22.1, 22.2).

ii) Harvest stage

When the samples were taken for recording dry matter production under Phytotron as well as under natural conditions, a notable enhancement in this parameter was recorded with the increase in rate of application of nitrogenous fertilizer. Blue green algal inoculation also brought about a significant effect on this parameter. Inoculation of pots with *Anabaena variabilis* resulted in highest plant dry matter production and lowest was observed under *Tolypothrix tenuis* inoculation in comparison to other biofertilizer strains examined. During harvest also the treatment combination N_2S_1 (100% N and *Anabaena variabilis* inoculation) resulted in

Table 22. Plant dry matter production

22.1 Influence of blue green algal inoculation on plant dry matter (g pot^{-1}) production at different levels of nitrogenous fertilizer under Phytotron conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	2.78	6.67	9.25	6.24	5.39	13.06	21.00	13.15
S_1	6.02	8.64	13.11	9.26	12.63	18.88	31.45	20.99
S_2	4.72	7.57	9.85	7.38	7.78	14.39	24.33	15.50
S_3	5.69	8.15	11.71	8.51	9.79	17.59	27.44	18.27
S_4	4.13	7.16	10.48	7.26	6.79	16.07	22.86	15.24
Mean	4.67	7.64	10.88		8.47	15.98	25.42	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.128	0.355			0.276	0.765	
Strain (S)		0.165	0.457			0.356	0.986	
N × S		0.285	0.789			0.617	1.709	

DMRT grouping for mean performance of plant dry matter production under phytotron conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_4	C	N_2S_2	C
N_2S_2	CD	N_2S_4	C
N_2S_0	DE	N_2S_0	D
N_1S_1	EF	N_1S_1	E
N_1S_3	FG	N_1S_3	EF
N_1S_2	GH	N_1S_4	FG
N_1S_4	HI	N_1S_2	GH
N_1S_0	IJ	N_1S_0	H
N_0S_1	JK	N_0S_1	H
N_0S_3	K	N_0S_3	I
N_0S_2	L	N_0S_2	J
N_0S_4	L	N_0S_4	JK
N_0S_0	M	N_0S_0	K

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 and N_2 represents 50% and 100% N levels as in Hoagland's medium
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

22.2 Influence of blue green algal inoculation on plant dry matter (g pot^{-1}) production under different levels of nitrogenous fertilizer under natural conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	2.65	6.75	12.35	7.25	9.37	20.64	33.69	21.23
S_1	6.23	10.75	44.41	20.47	15.8	31.65	47.56	31.67
S_2	4.40	7.45	17.23	9.69	14.16	23.61	39.86	25.88
S_3	5.45	9.72	25.23	13.47	15.41	28.74	43.02	29.05
S_4	3.53	8.48	13.47	8.49	10.87	25.84	37.63	24.78
Mean	4.45	8.63	22.54		13.12	26.10	40.35	

	Midcrop stage		Harvest stage	
	SE (m)±	CD (P=0.05)	SE (m)±	CD (P=0.05)
N level (N)	0.55	1.52	0.349	0.967
Strain (S)	0.71	1.97	0.450	1.247
N × S	1.22	3.38	0.780	2.161

DMRT grouping for mean performance of plant dry matter production under natural conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_2	C	N_2S_2	C
N_2S_4	D	N_2S_4	C
N_2S_0	D	N_2S_0	D
N_1S_1	DE	N_1S_1	D
N_1S_3	DEF	N_1S_3	E
N_1S_4	EFG	N_1S_4	F
N_1S_2	EFGH	N_1S_2	F
N_1S_0	FGH	N_1S_0	G
N_0S_1	FGHI	N_0S_1	H
N_0S_3	GHI	N_0S_3	H
N_0S_4	HI	N_0S_2	H
N_0S_2	HI	N_0S_4	I
N_0S_0	I	N_0S_0	I

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

maximum dry matter as was observed during midcrop stage (Tables 22.1, 22.2).

4.4.3.2 C content (g pot^{-1})

i) Midcrop stage

Under Phytotron as well as under natural conditions, there was a significant influence of nitrogenous fertilizer application on carbon content of the shoots as well as roots of rice plant. In comparison to that of uninoculated soil, blue green algal inoculation exhibited a notable influence on carbon content of shoot and root under both the growing conditions. Under Phytotron conditions, DMRT analysis indicated that the higher carbon content in shoots was observed in presence of *Anabaena variabilis* and *Nostoc muscorum* inoculation at 100% N. In roots the highest carbon content was observed under the treatment N_2S_1 in Phytotron conditions. Similar treatment combinations *i.e.* 100% N with *Anabaena variabilis* inoculation recorded highest shoot as well as root carbon content in the plant samples taken from natural conditions (Tables 23.1, 23.2, 23.3, 23.4, Fig.4).

ii) Harvest stage

During harvest stage also, a marked enhancement in C content was observed with increase in N application in shoot as well as in root under Phytotron and under natural conditions. There was a distinct influence of blue green algal inoculation on this attribute under Phytotron as well as under natural conditions. Inoculation of soil with *Anabaena variabilis* resulted in a highest C content in

Table 23. C content in plants

23.1 Influence of blue green algal inoculation on C content (g pot^{-1}) in shoot at different levels of nitrogenous fertilizer under Phytotron conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	0.363	1.00	2.04	1.13	0.50	1.40	2.79	1.56
S_1	0.933	1.72	2.93	1.86	1.22	2.35	4.53	2.70
S_2	0.647	1.21	2.29	1.38	0.64	1.62	2.99	1.75
S_3	0.827	1.41	2.92	1.72	0.79	2.04	3.52	2.11
S_4	0.573	1.33	2.54	1.48	0.52	1.96	2.73	1.74
Mean	0.67	1.33	2.54		0.73	1.88	3.31	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.024	0.065			0.043	0.116	
Strain (S)		0.031	0.084			0.055	0.149	
N × S		0.054	0.146			0.096	0.259	

DMRT grouping for mean performance of C content in shoot under Phytotron conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	A	N_2S_3	B
N_2S_4	B	N_2S_2	C
N_2S_2	C	N_2S_0	C
N_2S_0	D	N_2S_4	C
N_1S_1	E	N_1S_1	D
N_1S_3	F	N_1S_3	E
N_1S_4	FG	N_1S_4	E
N_1S_2	G	N_1S_2	F
N_1S_0	H	N_1S_0	FG
N_0S_1	HI	N_0S_1	G
N_0S_3	I	N_0S_3	H
N_0S_2	J	N_0S_2	H
N_0S_4	J	N_0S_4	H
N_0S_0	K	N_0S_0	H

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 and N_2 represents 50% and 100% N levels as in Hoagland's medium
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

23.2 Influence of blue green algal inoculation on C content (g pot^{-1}) in root at different levels of nitrogenous fertilizer under Phytotron conditions

N levels /Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	0.19	0.44	0.76	0.47	0.14	0.51	0.89	0.51
S_1	0.37	0.71	1.52	0.87	0.46	0.77	1.58	0.94
S_2	0.31	0.53	0.83	0.56	0.30	0.57	1.07	0.65
S_3	0.33	0.64	1.08	0.68	0.33	0.65	1.27	0.75
S_4	0.27	0.52	0.97	0.59	0.25	0.62	0.97	0.61
Mean	0.30	0.57	1.03		0.30	0.63	1.16	
	SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)		
N level (N)	0.030	0.081			0.011	0.030		
Strain (S)	0.039	0.105			0.014	0.038		
N × S	0.067	0.181			0.025	0.068		

DMRT grouping for mean performance of C content in root under Phytotron conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_4	BC	N_2S_2	C
N_2S_2	CD	N_2S_4	D
N_2S_0	CD	N_2S_0	E
N_1S_1	DE	N_1S_1	F
N_1S_3	DEF	N_1S_3	G
N_1S_2	EFG	N_1S_4	GH
N_1S_4	EFGH	N_1S_2	HI
N_1S_0	FGHI	N_1S_0	IJ
N_0S_1	GHIJ	N_0S_1	J
N_0S_3	GHIJ	N_0S_3	K
N_0S_2	HIJ	N_0S_2	K
N_0S_4	IJ	N_0S_4	K
N_0S_0	J	N_0S_0	L

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 and N_2 represents 50% and 100% N levels as in Hoagland's medium
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

23.3 Influence of blue green algal inoculation on C content (g pot^{-1}) in shoot at different levels of nitrogenous fertilizer under natural conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	0.41	1.05	2.19	1.22	1.85	5.55	12.74	6.71
S_1	1.03	1.66	9.07	3.92	4.06	8.64	17.57	10.09
S_2	0.67	1.19	3.29	1.72	3.36	4.59	15.10	7.68
S_3	0.84	1.59	4.48	2.30	3.81	8.97	16.40	9.73
S_4	0.59	1.33	2.39	1.43	1.98	7.76	14.41	8.05
Mean	0.71	1.36	4.28		3.01	7.10	15.25	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.073	0.202			0.253	0.683	
Strain (S)		0.094	0.249			0.327	0.883	
N × S		0.163	0.452			0.566	1.528	

DMRT grouping for mean performance of C content in shoot under natural conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	AB
N_2S_2	C	N_2S_2	BC
N_2S_4	D	N_2S_4	C
N_2S_0	D	N_2S_0	D
N_1S_1	E	N_1S_3	E
N_1S_3	E	N_1S_1	E
N_1S_2	EFG	N_1S_4	E
N_1S_4	EF	N_1S_0	F
N_1S_0	FGH	N_1S_2	FG
N_0S_1	FGH	N_0S_1	FG
N_0S_3	FGHI	N_0S_3	FG
N_0S_2	GHI	N_0S_2	GH
N_0S_4	HI	N_0S_4	H
N_0S_0	I	N_0S_0	H

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

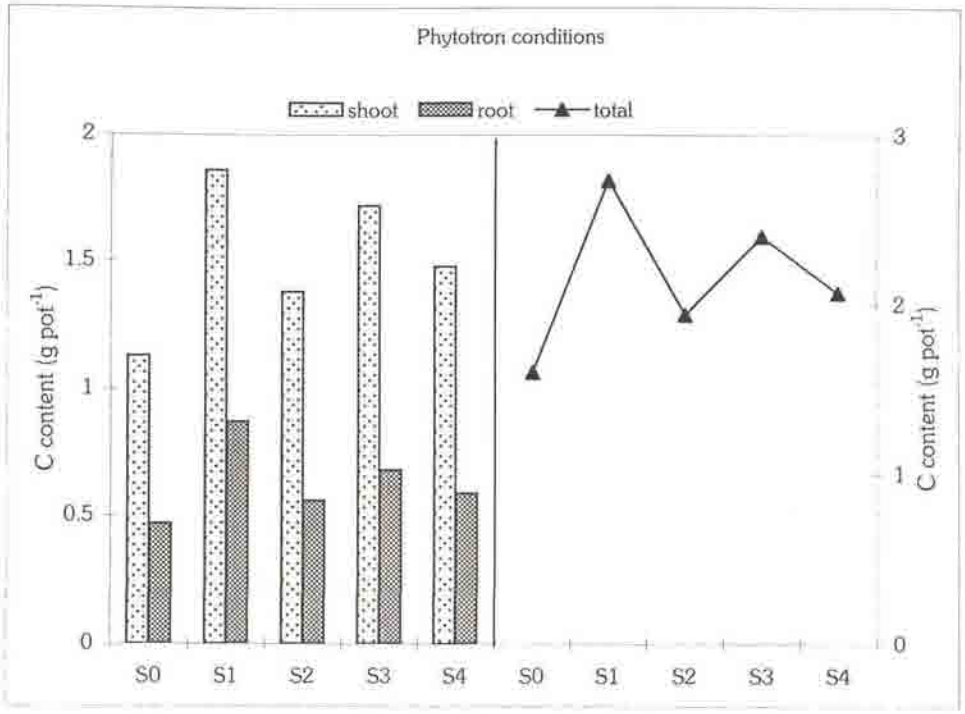
23.4 Influence of blue green algal inoculation on C content (g pot^{-1}) in root at different levels of nitrogenous fertilizer under natural conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	0.34	1.19	2.47	1.33	0.63	1.97	4.89	2.50
S_1	0.94	2.12	8.48	3.84	1.17	3.98	7.95	4.37
S_2	0.55	1.37	3.35	1.76	0.97	2.40	5.67	3.01
S_3	0.76	1.85	5.67	2.76	1.09	3.36	6.51	3.65
S_4	0.47	1.58	2.71	1.59	0.77	2.67	5.39	2.94
Mean	0.61	1.62	4.54		0.93	2.88	6.08	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.072	0.199			0.062	0.167	
Strain (S)		0.093	0.258			0.079	0.213	
N × S		0.162	0.449			0.138	0.373	

DMRT grouping for mean performance of C content in root under natural conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_2	C	N_2S_2	C
N_2S_4	D	N_2S_4	C
N_2S_0	DE	N_2S_0	D
N_1S_1	EF	N_1S_1	E
N_1S_3	FG	N_1S_3	F
N_1S_4	GH	N_1S_4	G
N_1S_2	GHI	N_1S_2	G
N_1S_0	HIJ	N_1S_0	H
N_0S_1	IJK	N_0S_1	I
N_0S_3	JKL	N_0S_3	I
N_0S_2	KL	N_0S_2	IJ
N_0S_4	KL	N_0S_4	IJ
N_0S_0	L	N_0S_0	J

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*



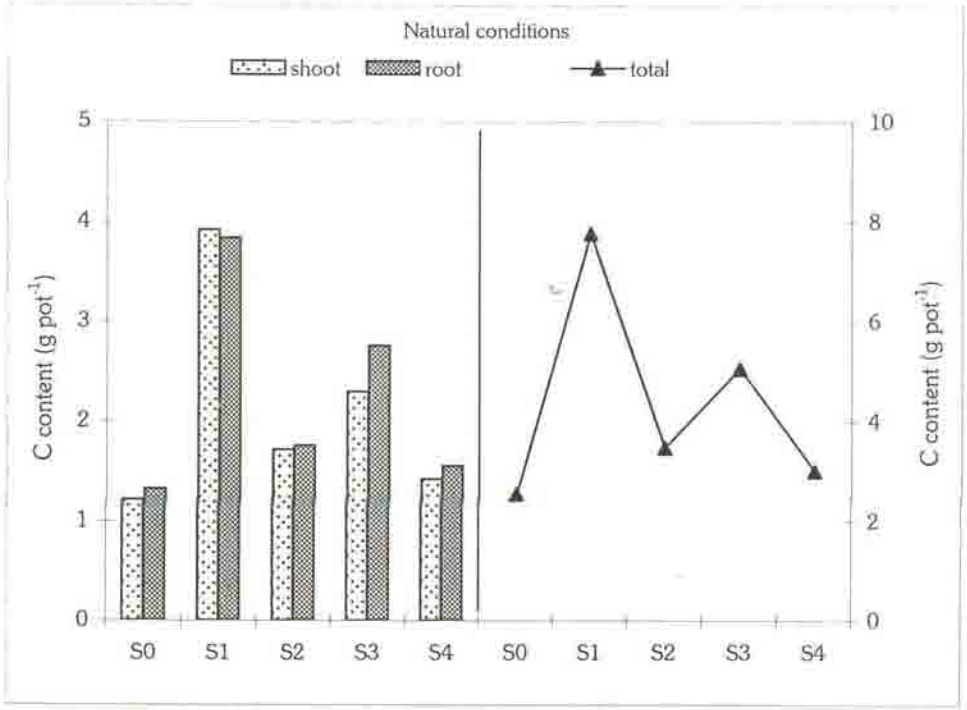


Fig.4 Influence of blue green algal inoculation on C content in rice crop during midcrop stage

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

shoot and root. In all the cases, the treatment combination N_2S_1 *i.e.,* 100% N in presence of *Anabaena variabilis* inoculation resulted in highest C content (Tables 23.1, 23.2, 23.3, 23.4, Fig.5).

4.4.3.3 N uptake (mg pot^{-1})

Percent N content or N concentration did not vary with different treatment combinations. However, N uptake differed due to significant influence of this treatment on dry matter production.

i) Midcrop stage

N uptake in shoot as well as root enhanced significantly under Phytotron as well as under natural conditions with the increase in the application of nitrogenous fertilizers. There was a notable influence of blue green algal inoculation on this attribute in comparison to control in shoot and root under Phytotron and natural conditions. The treatment combination having nitrogenous fertilizer @ 100% N in presence of *Anabaena variabilis* inoculation had most significant influence on this attribute in shoot and root under Phytotron as well as under natural conditions (Tables 24.1, 24.2, 24.3, 24.4, Fig.6).

ii) Harvest stage

N uptake enhanced significantly with the increase in N application rate under Phytotron as well as natural conditions in shoot and root. Blue green algal inoculation had a marked influence on this attribute with *Anabaena variabilis* inoculation being the best. The treatment combination *i.e.,* N_2S_1 and N_2S_3 for N uptake by shoot and N_2S_1 by root under Phytotron conditions were

Table 24. N uptake in plants

24.1 Influence of blue green algal inoculation on N uptake (mg pot^{-1}) by shoot at different levels of nitrogenous fertilizer under Phytotron conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	12.7	67.2	137.0	72.3	7.94	29.37	55.40	30.91
S_1	30.3	85.9	184.5	100.2	28.81	70.61	79.16	59.53
S_2	22.8	72.5	141.2	78.8	12.21	41.49	67.82	40.51
S_3	28.7	80.8	164.9	91.5	16.29	43.28	81.43	47.00
S_4	20.1	72.9	145.6	79.5	9.89	35.36	66.98	37.41
Mean	22.9	75.8	154.6		15.03	44.02	70.16	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		1.01	2.80			1.28	3.55	
Strain (S)		1.30	3.60			1.65	4.57	
N × S		2.25	6.23			2.86	7.92	

DMRT grouping for mean performance of N uptake by shoot under Phytotron conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_3	A
N_2S_3	B	N_2S_1	A
N_2S_4	C	N_2S_2	B
N_2S_2	CD	N_2S_4	B
N_2S_0	D	N_1S_1	B
N_1S_1	E	N_2S_0	C
N_1S_3	E	N_1S_3	D
N_1S_4	F	N_1S_2	D
N_1S_2	F	N_1S_4	DE
N_1S_0	F	N_1S_0	E
N_0S_1	G	N_0S_1	E
N_0S_3	GH	N_0S_3	F
N_0S_2	HI	N_0S_2	F
N_0S_4	I	N_0S_4	F
N_0S_0	J	N_0S_0	F

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 and N_2 represents 50% and 100% N levels as in Hoagland's medium
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

24.2 Influence of blue green algal inoculation on N uptake (mg pot^{-1}) by root at different levels of nitrogenous fertilizer under Phytotron conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	1.22	4.55	12.19	5.99	0.68	7.30	19.45	9.14
S_1	3.54	10.65	46.53	20.24	5.79	15.18	33.08	18.02
S_2	2.51	6.36	14.41	7.76	3.04	8.53	23.30	11.62
S_3	2.89	8.74	23.31	11.64	3.53	11.16	27.53	14.07
S_4	2.09	5.64	18.77	8.83	2.13	9.63	27.53	10.91
Mean	2.45	7.19	23.04		3.03	10.36	24.87	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		1.94	5.37			0.29	0.80	
Strain (S)		2.51	6.95			0.38	1.05	
N × S		4.34	12.02			0.66	1.83	

DMRT grouping for mean performance of N uptake by root under Phytotron conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_4	BC	N_2S_2	C
N_2S_2	BCD	N_2S_4	D
N_2S_0	BCD	N_2S_0	D
N_1S_1	BCD	N_1S_1	E
N_1S_3	CD	N_1S_3	F
N_1S_2	CD	N_1S_4	FG
N_1S_4	CD	N_1S_2	GH
N_1S_0	CD	N_1S_0	HI
N_0S_1	D	N_0S_1	I
N_0S_3	D	N_0S_3	J
N_0S_2	D	N_0S_2	J
N_0S_4	D	N_0S_4	JK
N_0S_0	D	N_0S_0	K

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 and N_2 represents 50% and 100% N levels as in Hoagland's medium
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

24.3 Influence of blue green algal inoculation on N uptake (mg pot^{-1}) by shoot at different levels of nitrogenous fertilizer under natural conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	15.51	43.18	98.5	52.39	18.6	62.8	119.2	66.8
S_1	37.12	70.96	397.7	168.6	34.0	97.3	152.0	94.4
S_2	25.46	46.84	142.5	71.61	24.1	61.5	126.6	70.8
S_3	31.24	60.73	199.0	96.98	28.8	91.1	136.1	85.4
S_4	21.17	54.74	105.9	60.60	17.7	65.2	116.9	66.6
Mean	26.09	55.29	88.72		24.6	75.6	130.1	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		4.92	13.6			1.93	5.35	
Strain (S)		6.35	17.6			2.49	6.90	
N × S		11.0	30.5			4.32	11.97	

DMRT grouping for mean performance of N uptake by shoot under natural conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_2	C	N_2S_2	BC
N_2S_4	D	N_2S_0	C
N_2S_0	DE	N_2S_4	C
N_1S_1	EF	N_1S_1	D
N_1S_3	FG	N_1S_3	D
N_1S_4	FGH	N_1S_4	E
N_1S_2	FGHI	N_1S_0	E
N_1S_0	FGHI	N_1S_2	E
N_0S_1	FGHI	N_0S_1	F
N_0S_3	GHI	N_0S_3	FG
N_0S_2	GHI	N_0S_2	FG
N_0S_4	HI	N_0S_0	G
N_0S_0	I	N_0S_4	G

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

24.4 Influence of blue green algal inoculation on N uptake (mg pot^{-1}) by root at different levels of nitrogenous fertilizer under natural conditions

N levels/ Strains	Midcrop stage				Harvest stage			
	N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean
S_0	3.35	13.96	47.72	21.68	1.6	8.1	17.9	9.27
S_1	8.12	21.51	144.3	57.97	3.00	15.3	29.5	15.9
S_2	5.67	13.77	58.6	26.02	2.3	9.0	20.1	10.5
S_3	7.32	18.73	94.6	40.23	2.8	12.8	20.9	12.2
S_4	4.44	16.41	46.0	22.29	1.8	10.3	20.6	10.9
Mean	5.78	16.88	78.53		2.30	11.1	21.8	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		1.61	4.46			0.36	1.00	
Strain (S)		2.08	5.76			0.47	1.30	
N × S		3.60	9.97			0.81	2.24	

DMRT grouping for mean performance of N uptake by root under natural conditions

Midcrop stage		Harvest stage	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	B	N_2S_3	B
N_2S_2	C	N_2S_4	B
N_2S_4	D	N_2S_2	BC
N_2S_0	D	N_2S_0	C
N_1S_1	E	N_1S_1	D
N_1S_3	EF	N_1S_3	E
N_1S_4	EFG	N_1S_4	F
N_1S_0	EFGH	N_1S_2	F
N_1S_2	EFGH	N_1S_0	F
N_0S_1	FGH	N_0S_1	G
N_0S_3	FGH	N_0S_3	G
N_0S_2	GH	N_0S_2	G
N_0S_4	H	N_0S_4	G
N_0S_0	H	N_0S_0	G

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

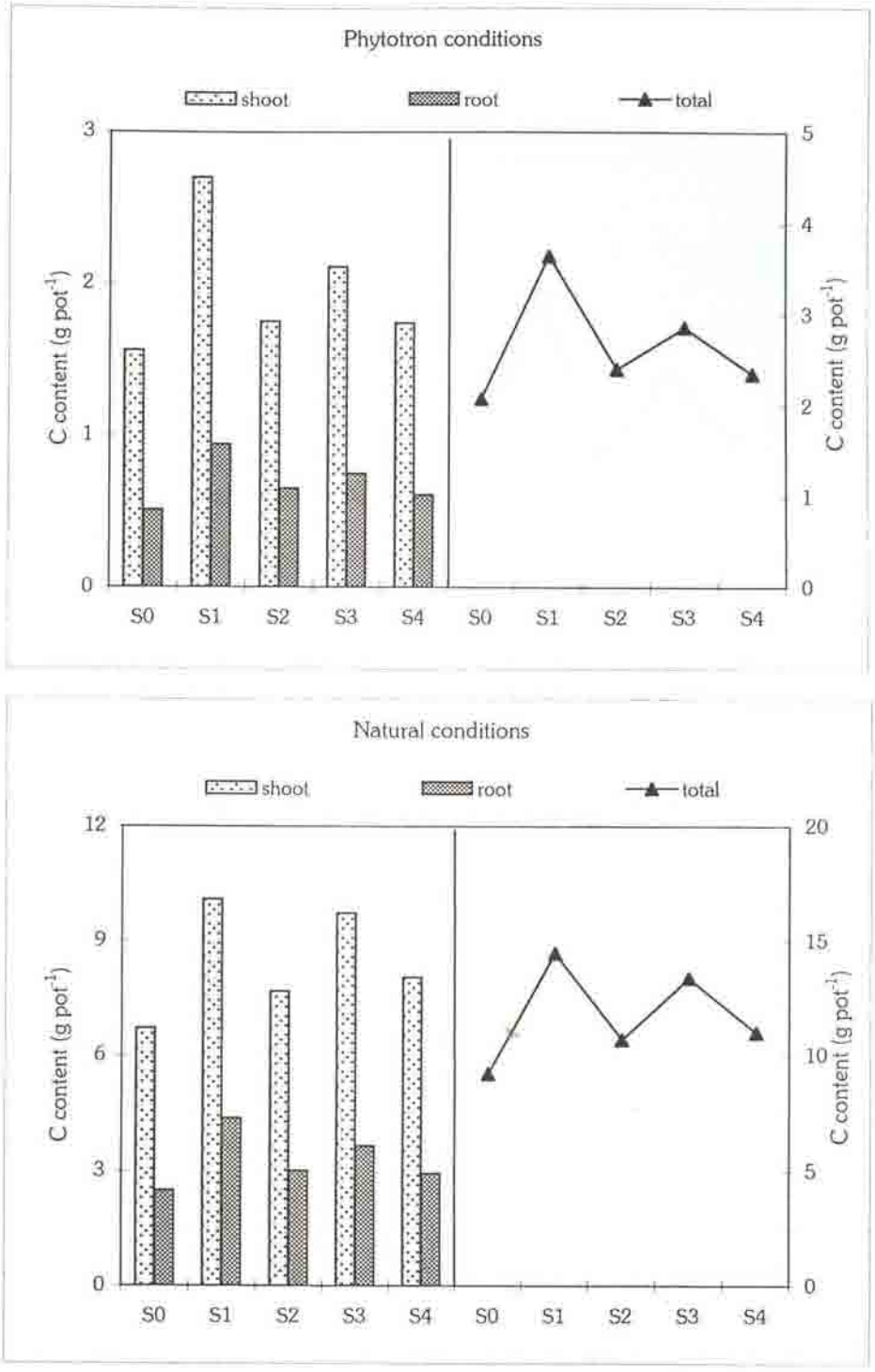


Fig.5 Influence of blue green algal inoculation on C content in rice crop during harvest stage

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

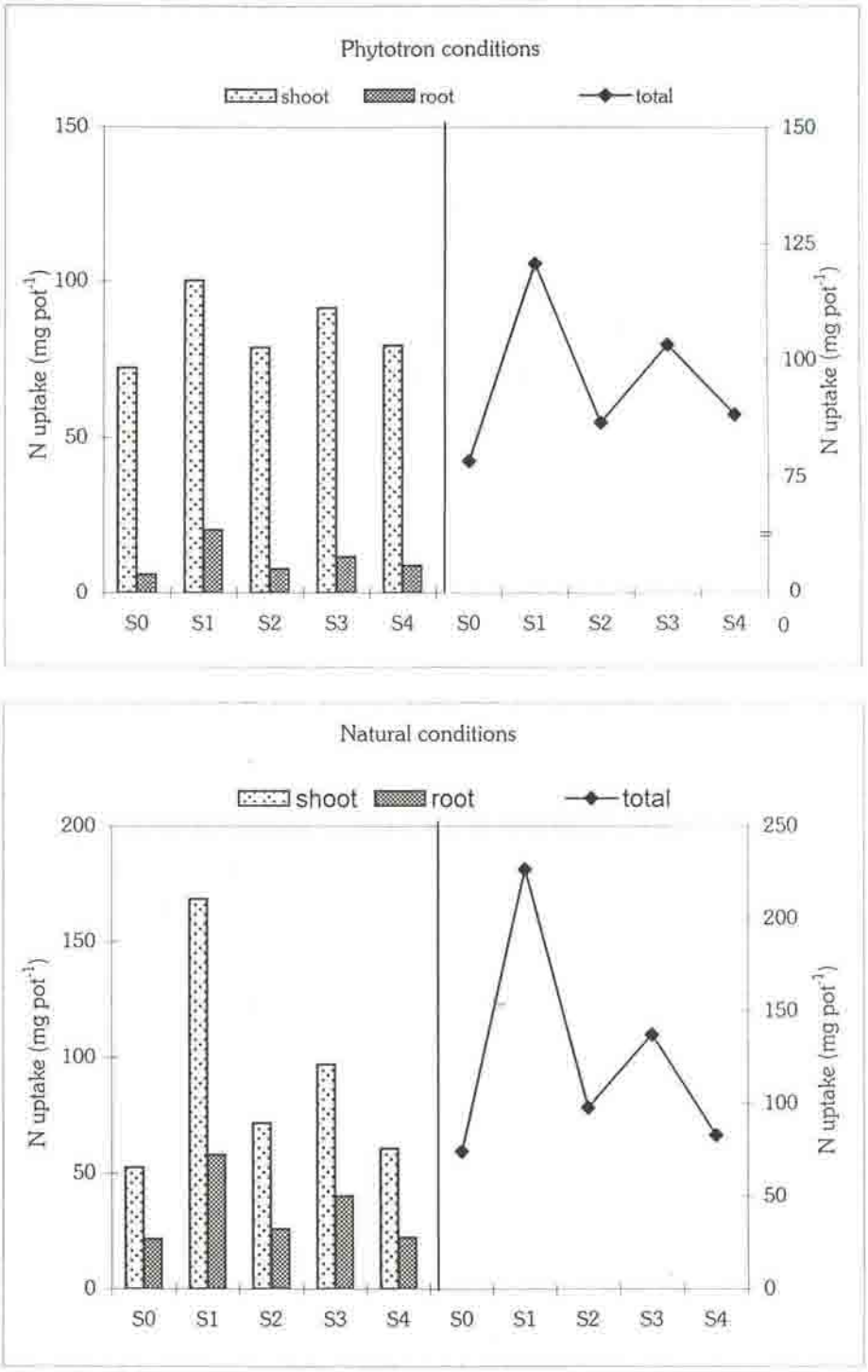


Fig.6 Influence of blue green algal inoculation on N uptake in rice crop during midcrop stage

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

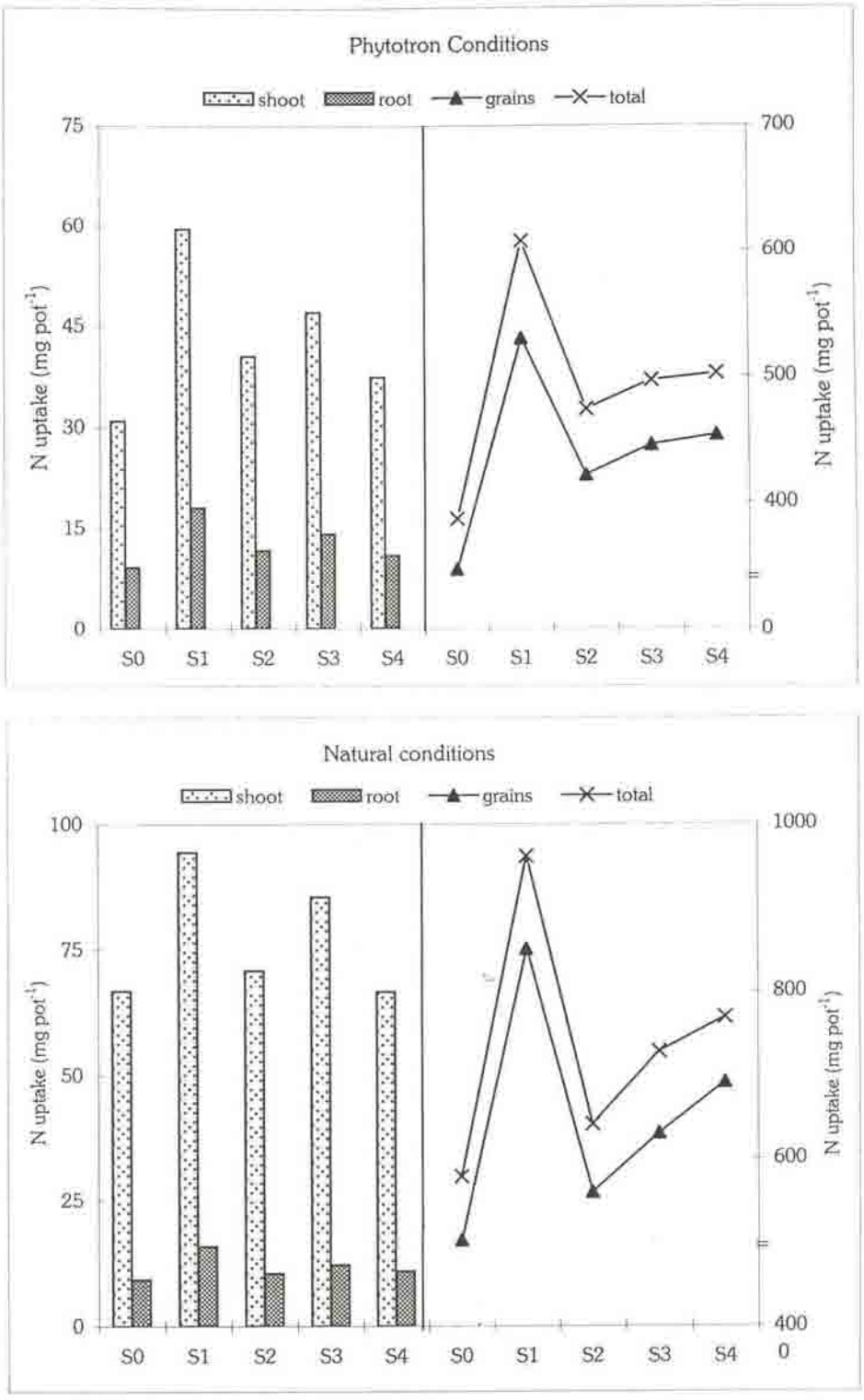


Fig.7 Influence of blue green algal inoculation on N uptake in rice crop during harvest stage

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

observed to be the best. However, N uptake by shoot and root under natural conditions was highest under the treatment where N was applied @ 100% N in the presence of *Anabaena variabilis* inoculation (Tables 24.1, 24.2, 24.3, 23.4, Fig.7).

4.4.3.4 Correlation analysis

When correlation coefficient (r) was analysed between soil parameters (available nitrogen, organic carbon and soil microbial biomass carbon) with plant parameters (N uptake and C content) during midcrop stage and harvest stage, it was found that available nitrogen and soil microbial biomass carbon correlated positively even at 1% probability with N uptake and C content in plants. The correlation between soil organic carbon with plant parameters however, was non-significant at both the stages of crop growth (Table 25).

4.4.3.5 Yield attributes

a) Panicle number per pot

There was a significant enhancement of panicle number per pot with the increase in the levels of nitrogenous fertilizer and the panicle number observed was more than two times at N_2 (*i.e.*, 100% N) than that observed at N_0 (0 N). Biofertilizer strains exhibited a marked influence on panicle number in Phytotron as well as natural conditions. Highest panicle number per pot was observed under the treatment N_2S_1 (100% N, *Anabaena variabilis*) and N_2S_4 (100% N, *Tolypothrix tenuis* inoculation) under both growing conditions of rice crop (Table 26.1, Fig.8).

Table 25 . Correlation co-efficient (r) between soil parameters (microbial biomass carbon, organic carbon and available nitrogen) and plant parameters (C content and N uptake)

Soil/Plant	Midcrop stage		Harvest stage	
	C content	N uptake	C content	N uptake
Microbial biomass carbon	0.76**	0.75**	0.92**	0.91**
Organic carbon	NS	NS	NS	NS
Available nitrogen	0.90**	0.88**	0.98**	0.96**

** denotes significant at 0.001p

Table 26. Yield attributes

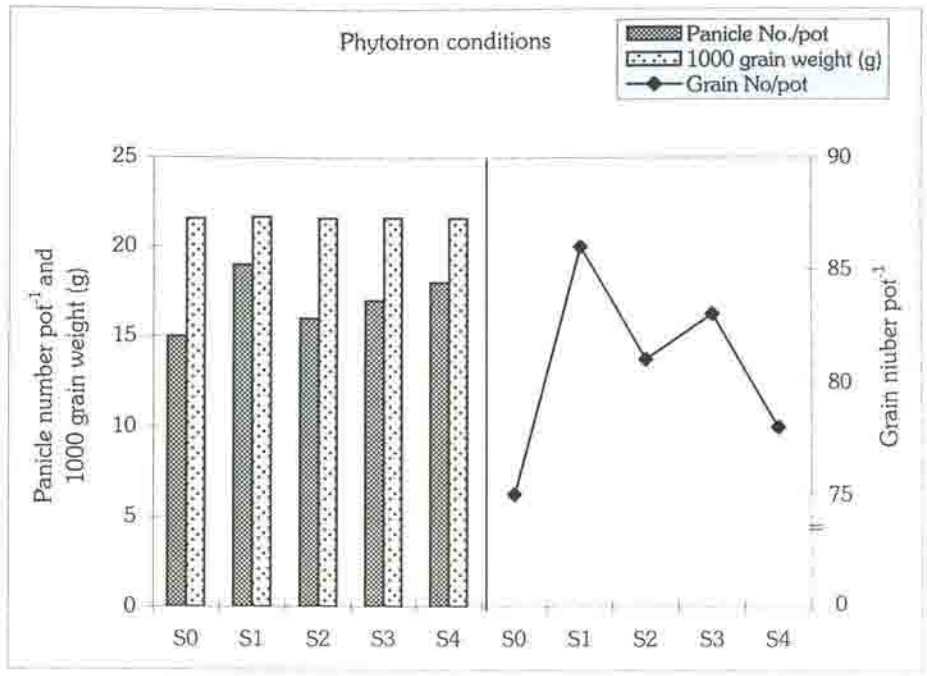
26.1 Influence of blue green algal inoculation on panicle number pot^{-1} under different levels of nitrogenous fertilizer

N levels/ Strains	Phytotron conditions				Natural conditions			
	N_0	N_1*	N_2*	Mean	N_0	N_1	N_2	Mean
S_0	9	14	22	15	7	13	19	13
S_1	12	21	24	19	10	21	25	19
S_2	10	17	22	16	8	13	20	14
S_3	11	18	22	17	10	16	20	15
S_4	10	20	24	18	7	17	25	16
Mean	10	18	22		8	16	22	
		SE (m)±	CD (P=0.05)			SE (m)±	CD (P=0.05)	
N level (N)		0.29	0.81			0.38	1.06	
Strain (S)		0.38	1.04			0.49	1.37	
N × S		0.65	1.80			0.86	2.37	

DMRT grouping for mean performance of panicle number pot^{-1}

Phytotron conditions		Natural conditions	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_4	A	N_2S_4	A
N_2S_3	AB	N_1S_1	B
N_2S_0	ABC	N_2S_2	B
N_2S_2	ABC	N_2S_3	B
N_1S_1	BC	N_2S_0	BC
N_1S_4	CD	N_1S_4	CD
N_1S_3	DE	N_1S_3	D
N_1S_2	E	N_1S_0	E
N_1S_0	F	N_1S_2	E
N_0S_1	FG	N_0S_1	F
N_0S_3	GH	N_0S_3	F
N_0S_2	HI	N_0S_2	FG
N_0S_4	HI	N_0S_0	G
N_0S_0	I	N_0S_4	G

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1* and N_2* represent 50% and 100% N levels as in Hoagland's medium
N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*



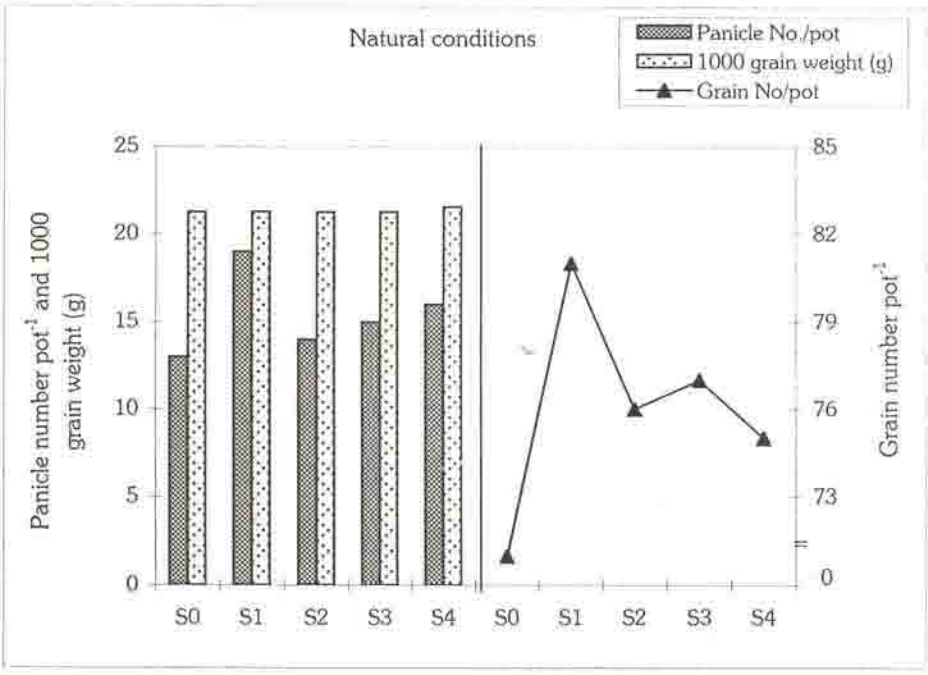


Fig.8 Influence of blue green algal inoculation on yield attributes of rice crop

S0-Without inoculation; S1-*Anabaena variabilis* ; S2-*Aulosira fertilissima* ; S3-*Nostoc muscorum* ; S4-*Tolypothrix tenuis* .

b) Grain number per panicle

This parameter also exhibited impressive enhancement with the application of nitrogenous fertilizer under Phytotron and natural conditions. Different biofertilizer strains exhibited a differential effect on this attribute. *Anabaena variabilis* showed highest influence in comparison with other biofertilizer strains tested. Treatment combination N_2S_1 *i.e.,* inoculation with *Anabaena variabilis* under 100% N showed the most significant impact on this parameter (Table 26.2, Fig.8).

c) Thousand grain weight

This attribute indicating the size of the grain remained more or less same under different treatment combinations in which different levels of nitrogenous fertilizer and different blue green algal biofertilizer strains were utilized (Table 26.3, Fig.8).

d) Nitrogen concentration (%)

Grain nitrogen concentration seems to be influenced with the application of nitrogenous fertilizer and biofertilizer strains. Increase in N level enhanced grain N concentration under Phytotron as well as natural conditions. Application of blue green algal strains revealed a little impact on this attribute. The inoculation with *Anabaena variabilis* showed little and insignificant effect on percent N content. Application of 100% N in presence of *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Tolypothrix tenuis* inoculation showed the best effect on this attribute analysed as DMRT grouping. However, under natural conditions, N_2S_1 treatment combination was the best followed by N_2S_3 which was *at par* with N_2S_4 treatment combination respectively (Table 26.4).

26.2 Influence of blue green algal inoculation on grain number panicle^{-1} under different levels of nitrogenous fertilizer

N levels Strains	Phytotron conditions				Natural conditions			
	N_0	N_1*	N_2*	Mean	N_0	N_1	N_2	Mean
S_0	65	75	84	75	62	71	81	71
S_1	76	90	92	86	71	84	87	81
S_2	72	85	86	81	68	76	84	76
S_3	74	88	87	83	68	79	83	77
S_4	68	78	88	78	65	77	85	75
Mean	71	83	87		66	77	84	

	SE (m)±	CD (P=0.05)	SE (m)±	CD (P=0.05)
N level (N)	0.26	0.71	0.30	0.84
Strain (S)	0.33	0.92	0.39	1.08
N × S	0.57	1.58	0.68	1.88

DMRT grouping for mean performance of grain number panicle^{-1}

Phytotron conditions		Natural conditions	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_1S_1	B	N_2S_2	B
N_1S_3	C	N_2S_4	B
N_2S_4	C	N_2S_3	B
N_2S_2	CD	N_2S_0	C
N_2S_3	CD	N_1S_1	D
N_1S_2	D	N_1S_3	D
N_2S_0	E	N_1S_4	E
N_1S_4	F	N_1S_2	E
N_0S_1	G	N_0S_1	F
N_0S_3	G	N_1S_0	F
N_1S_0	G	N_0S_2	G
N_0S_2	H	N_0S_3	G
N_0S_4	I	N_0S_4	H
N_0S_0	J	N_0S_0	I

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1* and N_2* represent 50% and 100% N levels as in Hoagland's medium
N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

26.3 Influence of blue green algal inoculation on thousand grain weight (g) under different levels of nitrogenous fertilizer

N levels / Strains	Phytotron conditions				Natural conditions			
	N_0	N_1*	N_2*	Mean	N_0	N_1	N_2	Mean
S_0	21.40	21.52	21.79	21.57	21.18	21.27	21.32	21.26
S_1	21.42	21.67	21.93	21.67	21.19	21.30	21.35	21.28
S_2	21.42	21.55	21.86	21.61	21.18	21.28	21.33	21.26
S_3	21.41	21.56	21.88	21.62	21.19	21.28	21.33	21.27
S_4	21.40	21.54	21.88	21.61	21.18	21.27	21.31	21.25
Mean	21.41	21.57	21.87		21.19	21.28	21.33	
N level (N)		NS				NS		
Strain (S)		NS				NS		
N × S		NS				NS		

DMRT grouping for mean performance of thousand grain weight

Phytotron conditions		Natural conditions	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_2	A	N_2S_2	A
N_2S_3	A	N_2S_3	A
N_2S_0	A	N_2S_4	A
N_2S_4	A	N_2S_0	A
N_1S_1	A	N_1S_1	A
N_1S_3	AB	N_1S_3	AB
N_1S_2	ABC	N_1S_2	ABC
N_1S_0	ABC	N_1S_0	ABC
N_1S_4	ABC	N_1S_4	ABC
N_0S_1	D	N_0S_1	BC
N_0S_2	D	N_0S_3	BC
N_0S_3	D	N_0S_0	BC
N_0S_0	D	N_0S_2	BC
N_0S_4	D	N_0S_4	C

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1* and N_2* represent 50% and 100% N levels as in Hoagland's medium
N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

26.4 Influence of blue green algal inoculation on N concentration (%) by grains under different levels of nitrogenous fertilizer

N levels/ Strains	Phytotron conditions				Natural conditions			
	N_0	N_1*	N_2*	Mean	N_0	N_1	N_2	Mean
S_0	0.98	1.34	1.53	1.28	1.83	2.40	2.65	2.29
S_1	1.18	1.44	1.59	1.40	1.95	2.64	2.73	2.44
S_2	1.29	1.37	1.55	1.40	1.86	2.47	2.64	2.32
S_3	1.18	1.38	1.58	1.39	1.87	2.49	2.69	2.35
S_4	1.17	1.39	1.57	1.38	1.84	2.46	2.68	2.33
Mean	1.16	1.38	1.57		1.87	2.49	2.68	
		SE (m)±	CD @ 0.05p			SE (m)±	CD @ 0.05p	
N level (N)		0.015	0.041			0.005	0.014	
Strain (S)		0.019	0.051			0.006	0.016	
N × S		0.034	0.092			0.010	0.027	

DMRT grouping for mean performance of N concentration by grain

Phytotron conditions		Natural conditions	
Treatment combination	Group	Treatment combination	Group
N_2S_1	A	N_2S_1	A
N_2S_3	A	N_2S_3	AB
N_2S_4	A	N_2S_4	AB
N_2S_2	A	N_2S_0	B
N_2S_0	AB	N_2S_2	B
N_1S_1	BC	N_1S_1	B
N_1S_4	C	N_1S_3	C
N_1S_3	CD	N_1S_2	C
N_1S_2	CD	N_1S_4	C
N_1S_0	CD	N_1S_0	D
N_0S_2	D	N_0S_1	E
N_0S_1	E	N_0S_3	F
N_0S_3	E	N_0S_2	F
N_0S_4	E	N_0S_4	F
N_0S_0	F	N_0S_0	F

DMRT Grouping indicated by same alphabetic notations denotes non significant difference in mean values.
DMRT grouping in descending order with highest denoted by alphabet A
N_0 = Without nitrogen; N_1* and N_2* represent 50% and 100% N levels as in Hoagland's medium
N_1 = 60 kg N ha^{-1}; N_2 = 120 kg N ha^{-1};
S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

Discussion

5. DISCUSSION

Increasing concern about the long-term productivity of agroecosystems has emphasised the need to develop management strategies that maintain and protect soil resources. This is directly related to maintaining the quantity of soil organic matter which is an important component of soil productivity. Soil and crop management practices such as cultivation, crop rotation, residue management and fertilization exert a considerable influence on level of C in the soil. Phototrophs also have a significant influence on soil fertility due to fixation of atmospheric nitrogen. Nitrogen fixation is especially important in flooded soils such as rice paddies where blue green algal are recognized as significant contributors to the overall nutrient balance. Substantial information is available on various aspects of these organisms especially in relation to their use as N-supplements in rice cultivation (Singh, 1961; Roger and Kulasooriya, 1980; Venkataraman, 1981). These organisms may contribute to the maintenance of soil fertility by fixing atmospheric nitrogen in wetland soil or in symbiosis with higher plants or these may aid in aggregation of dried soil by producing abundant gelatinous capsules or slimes (Flaibani *et al.*, 1989). In view of this, pot culture studies were conducted at National Phytotron Facility and National Centre for Conservation and Utilisation of Blue Green Algae (during *kharif* 2002) to analyze the influence of blue-green

algal biofertilizer strains on specific soil properties in relation to carbon and nitrogen content and their effect on rice productivity.

Growth and characterization of BGA biofertilizer strains under laboratory conditions

In the present investigation, unialgal biofertilizer strains of blue green algae namely *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Tolypothrix tenuis* were used. Morphological and cultural characteristics of blue green algal strains during exponential growth in nitrogen free medium confirmed the identification and the purity of the strains, as per the keys given by Desikachary (1959). Intergeneric variations were observed with respect to growth potential, pigment profile, nitrogen fixing ability and N assimilation related parameters in the blue green algal biofertilizer strains. The parameters examined revealed a typical sigmoid curve showing peak during the exponential active growth phase as has been reported in large number of blue green algal strains (Venkataraman *et al.*, 1974). Important metabolites assessed such as soluble proteins and total sugars were highest at 14^{th} day and 21^{st} day of incubation. Nitrogenase as well as nitrate reductase activity observed was highest at 14^{th} day of incubation followed by a slow and gradual decline. Glutamine synthetase enzyme exhibited highest activity during active growth phase (14^{th} day of incubation) and ammonia release was also maximum at 14^{th} day of incubation indicating thereby, active fixation of N and its excretion as NH^{+}_{4} by these biofertilizer strains during exponential growth.

Dry weight recorded was significantly different among the blue green algal strains examined. Dry weight (mg mL^{-1}) and specific growth rate were highest in *Anabaena variabilis*. Comparative analysis indicated that parameters like chlorophyll, soluble proteins, total sugars and nitrate reductase activity were highest in *Aulosira fertilissima* and the highest values for phycocyanin, allophycocyanin, total phycobiliproteins, nitrogenase activity on chlorophyll and protein basis and GS activity were recorded in *Nostoc muscorum*. Carotenoids and phycoerythrin were notably maximum in *Tolypothrix tenuis* (Tables 27.1 &27.2).

Blue green algae are known to assimilate nitrogen from atmospherically available nitrogen, nitrate, nitrite, ammonia, hydroxylamine, amino acids *etc.* (Holm-Hamsen, 1968). Nitrogen fixed into ammonia may be excreted as extracellular ammonia and this potential was highest in *Anabaena variabilis*. The ammonia may also be fixed into glutamine within heterocysts and this glutamine is transported to vegetative cells where it is utilized for the synthesis of glutamate (Thomas *et al.*, 1975; Rai *et al.*, 1984). Under conditions of ammonia limitation, most prokaryotes use a pathway consisting of glutamine synthetase and glutamate synthase to assimilate ammonia (Miflin and Lea, 1976). In cyanobacteria, this pathway is the major ammonia assimilatory route under nitrogen fixing condition (Wolk *et al.*, 1976; Stacey *et al.*, 1977). Such studies have indicated that different blue green algal strains showing high potential for specific parameters can contribute to

Table 27. Comparative mean values for different parameters

27.1 *Pigment content and soluble proteins amongst* blue green algal biofertilizer strains

Strains	Chlorophyll	Carotenoids	PC	APC	PE	PBS	Soluble proteins
S_1	7.37	0.146	8.96	2.86	7.55	19.37	34.63
S_2	8.42	0.108	2.11	1.97	2.09	6.17	46.04
S_3	6.55	0.139	9.34	8.32	11.14	28.8	28.23
S_4	5.25	0.338	8.92	5.66	11.79	26.37	31.48
CD (P=0.05)	0.784	0.042	0.073	0.799	1.27		1.63

PC: Phycocyanin; APC: Allophycocyanin; PE: Phycoerythrin; PBS: Total Phycobiliproteins
All units are expressed as μg mg^{-1} dry weight

27.2 Total sugars and N assimilatory parameters amongst blue green algal biofertilizer strains

Strains	Total sugars (μg mg^{-1} dry weight)	Nitrogenase activity (nmol C_2H_4 mg^{-1} chl h^{-1})	NR activity (μmol NO_2^- μg^{-1} protein)	GS activity (μmol γ-GH mg^{-1} protein 30 min^{-1})	Ammonia excretion (mmol NH_4^+ mL^{-1})
S_1	77.4	1136	41.37	41.75	1.743
S_2	99.0	1229	70.96	24.66	0.810
S_3	94.8	1548	55.36	82.60	0.730
S_4	89.4	1384	59.79	46.36	1.305
CD (P=0.05)	7.72	62.7	9.42	7.11	0.219

NR: Nitrate reductase; GS: Glutamine synthetase; GH: Glutamyl hydroxamate
S_1 : *Anabaena variabilis*; S_2 : *Aulosira fertilissima*; S_3 : *Nostoc muscorum*; S_4 : *Tolypothrix tenuis*..

crop productivity through indirect means such as improvement of soil properties or stimulation of beneficial flora in soil and their combined consortia would therefore, benefit the crop in multiple ways. In the present investigation, the variability observed with respect to various parameters analysed was in accordance with results obtained from earlier studies conducted at NCCUBGA, IARI (Dhar *et al.*, 2000; Mishra *et al.*, 2001). Characterization of blue green algal strains has also been carried out for specific parameters in relation to their utilization in value addition (Garcia-Pichel and Castenholz, 1991).

Pot culture studies using BGA biofertilizer strains

Rice crop was raised under pot culture conditions in peat under Phytotron conditions, and in soil under natural conditions during *kharif* 2002 to understand the role of blue green algal biofertilizer strains on C and N content and rice productivity. The success of rice production in the tropics and subtropics depends upon an efficient and economic supply of nitrogen, an element required in the largest quantity, in comparison with other essential ones. Blue green algal strains can fix atmospheric nitrogen into ammonia, which can be taken up by rice crop. The N use efficiency of fertilizer sources in rice is low because of the loss from soils through various chemical and biochemical processes.

Blue green algal biofertilizer strains were taken alone or in combination with 3 levels of nitrogenous fertilizer. The comparison of results with control (uninoculated), -N and +N treatments in rice

variety Basmati-1 provided a considerable amount of information. Unlike chemical N fertilizers, blue green algae neither contaminate the environment nor consume the photosynthate of rice plants (Liu, 1979). These organisms are also known to bring about important changes in the physical, chemical and biological properties of the soil during their active growth and after their death.

Abundance of microbial flora

Inoculation of soil with BGA strains had a profound effect on specific properties under Phytotron conditions. The highest algal population was recorded with the application of *Anabaena variabilis* in the presence of 100% N (given as in Hoagland solution). Under uninoculated conditions, algal population was not detected in peat, in which rice crop was raised.

However, under natural conditions, different components of microbial flora were examined in soil. There was an enhancement in algal population with the application of blue green algal strains thereby, indicating the survival and establishment of inoculated and introduced strains during midcrop and harvest stage of crop growth period of 170 days. Total algal population ranged from 11.36 under uninoculated condition to 15.49 CFU x 10^3 g^{-1} under *Anabaena variabilis* treatment during midcrop stage. During harvest, the highest algal population (5.08 x 10^3 CFU g^{-1}) was observed when the pots were inoculated with *Tolypothrix tenuis*. It was found out that during midcrop, inoculation with *Tolypothrix tenuis* under 120 kg N ha^{-1} recorded highest algal CFU and during harvest the

treatment combination *i.e.* inoculation with *Anabaena variabilis* at zero level of nitrogenous fertilizer showed highest CFU. Earlier studies have indicated a negative correlation between the concentration of inorganic nitrogen fertilizer and blue-green algal abundance (Quesada and Fernandez-Valiente, 1996). The increased nitrogen level may help in better proliferation of non-heterocystous blue green algae. Under such conditions, heterocystous forms without heterocysts may also develop. Whitton (2000) emphasised that combined use of direct counts with plating technique is essential for accurate cell counts of soil blue green algae.

Sometimes grazing can be an important factor for the establishment of algal inocula. The results of our studies on the survival of BGA strains was in agreement with earlier studies where a number of blue green algal species were inoculated onto flooded brown earth silt loam (Rao and Burns, 1990). However, in their studies, by day 147, the number of introduced BGA was no different than those of non-inoculated soils. It may be mentioned that the soil moisture content is usually critical for survival of phototrophs (Drew and Anderson, 1977). The greater abundance of blue green algae in the preflood and flooded periods may be due to the high calcium content of soil and water (Whitton *et al.*, 1988a,b) in view of the tendency for blue green algae to be favoured by calcareous environments. It may be possible that environmental factors such as water content and conductivity affect

distribution of soil algae and more frequent sampling is essentially required to analyze the seasonal variation.

The soil bacterial, fungal and actinomycetes population was enhanced with the application of nitrogenous fertilizer and further stimulated when BGA inoculum was provided. Highest bacterial population was observed in the soil sample inoculated with *Anabaena variabilis* during midcrop as well as during harvest. Fungal population analysed was highest in the pots inoculated with *Tolypothrix tenuis* during midcrop stage. However, during harvest, highest fungal population was recorded when the pots received *Nostoc muscorum* as the inoculum. During midcrop stage, the highest actinomycetes population was recorded in presence of *Nostoc muscorum* and during harvest, *Anabaena variabilis* inoculation resulted in highest actinomycetes population. Comparative analysis carried out indicated that the total microbial counts were highest when pots received *Anabaena variabilis* as inoculum during midcrop stage and during harvest stage. The data analysed clearly indicated that the bacterial population contributed significantly to the total microbial flora. This revealed that even if artificial inoculation in the form of blue green algal application is given, maximum contribution to total microbial flora is through bacterial population only.

BGA are also known to release carbonaceous metabolites, which may have stimulating effect on heterotrophic microflora. If certain aerobic and microaerophilic nitrogen fixing organisms such

as *Azotobacter*, *Azospirillum*, *Pseudomonas* and others are inoculated along with blue green algae, they can make use of excess oxygen as well as the easily oxidisable carbonaceous metabolites liberated by BGA for their respiration and as a source of energy respectively (Mandal *et al.*, 1999). This process may compensate for the loss of any nitrogen through denitrification and also augment atmospheric nitrogen addition to the soil. Much information is not available regarding the associated changes in soil micro flora following inoculation with blue green algae, although Venkataraman (1975) indicated that the number of other microorganisms is affected. In a pot culture experiment with *Tolypothrix tenuis* as the inoculant, Ibrahim *et al.* (1971), observed an increase in total microbial population, especially the number of nitrifiers, namely the genera *Azotobacter* and *Clostridium*, when a mixture of blue green algal inocula was used (Rao and Burns, 1990). It was observed that there was a 8 fold increase in bacterial numbers after 13 weeks of inoculation and the increase was only 2-8 fold after 21 weeks. Similarly, Rogers and Burns (1994) recorded 500 fold, 16 fold and 48 fold increase in bacteria, fungi and actinomycetes population respectively in the treatments which were inoculated with *Nostoc muscorum* over non-inoculated control. None of these workers however, indicated any additional benefits gained due to such increased population of beneficial microflora. The success of blue green algae highlights the advantage of using a phototrophic system in preference to heterotrophic inoculant

because the phototrophic inoculant will not compete for soil carbon or nitrogen resources and C limitation is one of the major reasons why heterotrophic soil inoculants fail to become established (Wessendorf and Lungens, 1989). The increased flush of heterotrophic microbial population in the inoculated soils were probably in response to additional carbon and energy source under inoculated conditions. Increase in energy source may also be due to the microbial polysaccharides (Lynch and Bragg, 1985). Further, increase in available nitrogen under the inoculated soils might have also stimulated indigenous soil microorganisms. Anderson and Gray (1991) in their studies have demonstrated that the nutrient status of the soil with specific reference to N and P may determine the mineralization of carbon and resulting size of the microbial biomass. Furthermore, microbe-soil structure relationship is reciprocal and increase in heterotroph numbers may be in response to the improved aggregate stability. Aggregate stability is known to improve soil aeration and solute transport thereby, creating more conducive environment for soil microorganisms (Lynch and Bragg, 1985; Rogers and Burns, 1994).

Influence of BGA biofertilizer strains on specific soil parameters

The relationship between nitrogen fixing microorganisms and physical and chemical characteristics of sediments have been described earlier (Quesada and Fernandez-Valiente, 1996, Quesada *et al.*, 1997). Our investigation clearly indicated that

inoculation of soil with blue green algae can bring about appreciable changes in some of the electrochemical and chemical properties of soil particularly in relation to redox potential, availability of nitrogen and other nutrients, which might influence growth, and productivity of rice crop. This is in accordance with the studies undertaken by other workers (Saha *et al.*, 1982).

Specific soil parameters such as pH and EC were not influenced markedly due to blue green algal inoculation. However, redox potential was highest with *Nostoc muscorum* inoculation during midcrop and with *Anabaena variabilis* inoculation during harvest. Redox potential enhancement with blue green algal inoculation contradicts the earlier report in which it was shown that the addition of decomposable organic matter to flooded rice soil enhances the fall of redox potential and thereby, accentuates the redox potential in soils (Ponnamperuma, 1972). Addition of BGA has shown to bring about changes in oxidizable carbonaceous materials, thus making the soil more reducing (Saha *et al.*, 1982).

Blue green algae are aerobic photosynthetic organisms and evolve oxygen during photosynthesis through photosystem II. As a result, when they grow in the rice fields they make the standing water highly oxygenated (Harrison and Aiyer, 1913). The profuse growth of BGA may increase the oxygen concentration which sometimes, may tend to reach 10-12 $\mu g\ g^{-1}$ (Lakshmanan *et al.*, 1994). This is an important beneficial effect in rice fields where continuous waterlogging creates intense reducing conditions with

redox potential value falling below -200 mV (Ponnamperuma, 1972). Inoculation of rice fields with BGA is helpful in regulating the formation of toxic substances by maintaining the redox potential at relatively high level and high oxygen tension due to algal photosynthesis which facilitates the oxidation of reduced components. The oxygen released by BGA may accelerate losses of nitrogen from the soil by nitrification and denitrification (Patrick and Reddy, 1978), however, this may be compensated by the beneficial effects of blue-green algae through N accretion (Mandal *et al.,* 1999).

Proliferation of BGA can be evaluated in terms of chlorophyll, a commonly measured growth attribute and parameters estimating reducing-oxidising activities in soil. Nitrogen, an important essential nutrient is a key factor for lowland rice production. Soil nitrogen pool is believed to be maintained through the biological nitrogen fixation (Kundu and Ladha, 1995; Roger and Ladha, 1992) and fertilizer N. But the efficiency of N fertilizer in rice crop is very low due to large N losses from flooded soil (De Datta and Buresh, 1989). Soil nitrogen is the principal source of nitrogen for rice and more than 50% of N used by rice receiving N fertilizers is derived from native soil nitrogen (Broadbent, 1984). Among indigenous nitrogen fixers in rice fields, blue green algae, are the main contributors to nitrogen fixation (Rogers and Ladha, 1992). Their nitrogen fixing potential and agronomic importance has been recognized particularly in relation to rice cultivation (Watanabe *et al.,* 1977; Singh, 1977,

1979; Liu Chung-Chiu, 1979; Dao and Tran, 1979). BGA, the photoautotrophic prokaryotes that are frequently considered as predominant diazotrophs in wetland rice systems, play a positive role in the sustenance of the nitrogen status and inherent fertility of rice fields (De, 1939).

At midcrop stage, the highest chlorophyll and dehydrogenase activity in soil samples were observed with *Tolypothrix tenuis* inoculation under 120 kg N ha^{-1} During harvest, the soil chlorophyll and dehydrogenase activity studied were highest when pots were inoculated with *Anabaena variabilis* at similar levels of N fertilizer. However, it is difficult to distinguish chlorophyll *a* from pheopigments, which are the degradation products of chlorophyll *a*. consequently, the pigment concentration may be overestimated (Tsujimura *et al.,* 2000). If we extract chlorophyll *a* from the soil properly, the biomass estimated by pigment extraction techniques can reflect the quantity of active cells at the time of sampling since resting cells have little chlorophyll (Coleman, 1983). The direct microscopic examination technique introduced by Tchan (1952) may resemble pigment extraction as it reflects active cells at the time of sampling. Dor and Danin (1996) examined chlorophyll *a* of deserts crusts after being wetted with distilled water and incubated for one week. The dehydrogenase activity seemed to follow the trend reported for total microbial biomass as reported in other studies (Chander *et al.,* 1997).

Highest nitrogenase activity was recorded during midcrop stage when soils received *Nostoc muscorum* as inoculum under 60 kg N ha^{-1}. During harvest, however *Anabaena variabilis* (at 60 kg N ha^{-1}) inoculation recorded maximum nitrogenase activity. *In situ* measurement of nitrogen fixation through acetylene reduction assay confirmed the stimulatory effect of blue green algal inoculation on nitrogenase activity and slight inhibitory effect of higher level of nitrogenous fertilizer *i.e.* 120 kg N ha^{-1}. These parameters were seen to increase with the application of blue green algal biofertilizer strains in comparison with control conditions. At N_1 (60 kg ha^{-1}), the nitrogenase activity appeared to be enhanced during midcrop as well as harvest stage. Enhancement in the algal population corresponded with the increase in nitrogenase activity where the soils were artificially inoculated with blue green algal strains.

Rowell *et al.* (1977) observed that increasing the level of nitrogenous fertilizer decreased nitrogenase activity, which is in accordance with our present study and nitrogen fixation reduced considerably in the presence of combined nitrogen. Lee *et al.* (1977) found that from 4-6 weeks after transplanting, ARA in the rhizosphere of rice cv. IR 26 increased to reach a maximum value at the heading stage, followed by a rapid decrease. This is similar to our observations in the present investigation in which maximum activity was observed during midcrop stage. The estimate of nitrogen fixed in sites, where no blue-green algal growth was visually apparent, ranged from 0.023 to 75.5 kg N ha^{-1} yr^{-1}

(Quesada *et al.*, 1997) when blue-green algal blooms were present, nitrogen fixation contributed 2 kg N ha^{-1} day^{-1} and nitrogenase activity assayed at midday was from one to three orders of magnitude higher in BGA covered soils in comparison to bare soil. Whole field estimates of N_2 fixed by blue green algal communities ranged from 1.0 to 10.2 kg N ha^{-1} (Rother and Whitton, 1989). Although indigenous algal flora has a greater competitive ability over the inoculated strains, the inoculum applied on the soil surface might benefit from more light and grow better than the indigenous flora that is mixed with soil, as growth and the germination of these organisms is generally photo dependent (Reddy, 1983).

Isotopic experiments have revealed that N fixed by these organisms may replenish soil nitrogen pool, which is the main source of nitrogen for rice and is readily available to the plant. Therefore, reasonable utilization of biological nitrogen in combination with inorganic nitrogen fertilizer would certainly allow significant reduction in N fertilizer input without loss of productivity, besides the ecological benefit it may represent for the ecosystem. Therefore, if algae could be cultured cheaply by harvesting unwanted algal blooms, these could prove to be a remarkably cost effective fertilizer supplement and the benefits from such treatments may be also due to other metabolic activities other than BNF.

Photoautotrophs present on the surface of soil may assimilate CO_2 present in the vicinity; resulting in the decrease in CO_2 pressure. As a result, CO_2 from air is diffused into the soil. The

production of CO_2 in soils results from oxidation of soil organic matter by heterotrophs and respiration of plant roots. The release of CO_2 from the soil is a major flux in the global C cycle, which is largely fuelled by plant production (Schlesinger, 1997). A considerable part of the CO_2 being assimilated by photoautotrophs comes from the soil. Some parts of the assimilated CO_2 may return to the soil in the form of algal cells etc. while the major part of organic C in soil is considered to have originated from the soil carbon because organic matter photosynthesized in flood water community is one of the important organic matter inputs to paddy soil (Takai and Wada, 1966). The CO_2 supply from the atmosphere may be significant when CO_2 concentration in floodwater is not enough to support vigorous photosynthetic activity. So floodwater plays an important role in the dynamics of CO_2 in the paddy field ecosystem.

Microbial biomass carbon was enhanced with the application of blue green algal strains in comparison to control conditions and maximum was recorded under *Anabaena variabilis* inoculation during midcrop as well as during harvest. It is worthwhile to point out that the percent contribution by microbial biomass carbon to the total organic carbon is only 1 to 5%. This amount may be insignificant in comparison to large proportion of organic carbon contributed by multiple types of flora and fauna and plant / animal debris available in the soils. Microbial biomass carbon was maximum with *Tolypothrix tenuis* and *Anabaena variabilis* inoculation at 120 kg

N ha^{-1} (Table 28). Over short periods, changes in microbial biomass carbon can be a sensitive index of changes in the content of soil organic matter (Powlson *et al.*, 1987). Much information is available on the role of microbial biomass carbon in soils under different management practices (Ross, 1990; Chander *et al.*, 1997) or different moisture regimes (Salinas-Garcia *et al.*, 1997). Microbial biomass carbon decreased during harvest time in the present investigation, which may be attributed to temporary drought experienced during harvest time. This parameter has been reported to decrease from planting time to flowering time and then increase at harvest to values similar to those at planting (Salinas-Garcia *et al.*, 1997). The size of microbial biomass may be governed by various management practices such as crop rotation, cultivation, organic amendments, fertilization and crop residue management (Mc Gill *et al.*, 1986). Marked seasonal variation in the amount of soil microbial biomass carbon has been observed from winter to summer with the studies suggesting that the carbon in soil microbial biomass may act as source of CO_2 released from the soil. Contrasting seasonal variability of soil CO_2 efflux and microbial biomass carbon suggest that CO_2 released from the soils is mainly a turn over product of soil microbial biomass carbon (Piao *et al.*, 2000a).

During midcrop, soil organic carbon was not significantly influenced. However, at harvest stage, there was an enhancement in organic C content with the application of BGA biofertilizer

Table 28. Influence of blue-green algal inoculation on specific soil parameters (Microbial biomass carbon, Organic carbon and Available nitrogen)

Strains	Microbial biomass carbon		Organic carbon		Available nitrogen	
	MS	HS	MS	HS	MS	HS
S_0	33.63	13.67	4660	5320	85.50	82.27
S_1	79.15	49.36	5740	12070	95.45	95.00
S_2	58.10	37.07	5900	6480	89.09	87.73
S_3	67.55	37.66	6360	7290	93.18	91.36
S_4	65.88	42.18	5270	6790	87.72	88.64
CD (P=0.05)	4.66	3.19	NS	530	2.7	3.08

MS= Midcrop stage; HS= Harvest Stage
All units are expressed as mg kg^{-1}

strains, when pots were inoculated with *Anabaena variabilis* at 120 kg N ha^{-1}. It must be emphasized that there was a notable enhancement in this parameter during harvest in comparison to midcrop stage of crop growth. Organic C production and chemical changes due to photosynthetic activities by autotrophic communities may greatly influence the fertility of rice soils. Information on this parameter regarding inoculation of soil with blue-green algae is rather scanty and higher air temperature under tropical conditions may stimulate turnover rates of soil microbial biomass carbon (Piao *et al.*, 2000a, b). A build up of organic matter, which may cause enhancement in organic C due to algal inoculation in soil has been claimed by earlier workers (De and Sulaiman, 1950; Mandal *et al.*, 1999). Osmanova (1979) also attributed the accumulation of organic matter due to growth of blue green algae using ^{15}N. Nekrasova and Aleksandrova (1982) confirmed that algal biomass contributed significantly to humus formation in soils despite the absence of typical lignin in them. All these studies have indicated that under favourable conditions a good algal bloom in rice fields yield on average about 6-8 t of fresh biomass (Roger and Kulasooriya, 1980; Roger *et al.*, 1987). The persistence of such biomass in soils as organic matter depends upon its decomposability and some algae may be decomposed quickly while others may last longer (Watanabe and Kiyohara, 1960). The differing susceptibility of algae to decomposition is related to the relative biodegradability of cell wall compounds and their physiological growth stages (Gunnison and

Alexander, 1975). Decomposability of *Anabaena* in soil is reported to be faster than other BGA and algal biomass rich in akinetes does not decompose easily in comparison to other vegetative cells (Mandal *et al.*, 1999). However, conclusive evidence on the rate of building up of biomass and its benefits can be achieved only from long-term inoculation studies. Most of the organic carbon fixed by blue algae might be lost from the soil due to rapid biochemical oxidation at high temperature prevailing during summer months. However, data from long-term experiments with blue green algae are lacking (Ventura and Watanabe, 1993). The results from the present study undertaken have clearly indicated that the soil rich in organic carbon have higher algal populations. The ratio of organic carbon and available nitrogen enhanced with the application of blue green algal inoculum and accordingly an increase in the algal counts were also noticed. The increase in soil carbon may be also influenced by aggregate stability, which may be attributed to the polysaccharides released by cyanobacteria. Although polysaccharides are considered to be an important factor in soil aggregation (Benzig-Purdie and Nikrforuf, 1989), some workers have suggested that it may not be due to the direct effect of polysaccharides, but through the products of their microbial degradation (Martens and Frankenberger, 1992). In this context, it should be mentioned that the occurrence of polyphenolic and hydrophobic metabolites may also play a significant role (Rogers and Burns, 1994). Elliott (1986) however, noted that it is the less highly processed organic matter such as

polysaccharides, which is lost during cultivation. Changes in microbial biomass carbon/soil organic carbon ratio reflects the input of organic matter to soils, efficiency of microbial incorporation, carbon losses from the soils and the stabilization of organic carbon by the soil mineral fractions (Sparling, 1992).

There is extensive literature available on ability of many species of blue green algae to fix N_2 and subsequent addition of this N to the ecosystem (Roger and Watanabe, 1986). Roger and Kulasooriya (1980) regard 30 kg N ha^{-1} yr^{-1} as satisfactory value when environmental conditions are favourable. The available N of rice soils is reported to increase considerably by the growth of nitrogen fixing blue green algae (De and Mandal, 1956; Singh, 1961; Stewart *et al.,* 1968; Watanabe and Yamamoto, 1971; Venkataraman, 1972 ; Singh, 1978; Saha *et al.,* 1982). In the present investigation, available nitrogen was enhanced with the application of blue green algal biofertilizer strains and was highest during midcrop as well as harvest stage when the pots received *Anabaena variabilis* inoculation and the lowest available nitrogen was observed in the uninoculated control pots.

The variation in the magnitude of the level of available N at different periods may be the result of many interacting processes including mineralization-immobilization and losses through various means. Sometimes, immobilization of the rapid hydrolysis product of urea by growing blue green algal cells, and suppression of nitrogen fixing activity may cause of reduction in N content of soil.

This negative influence of inoculation may become less significant at later stages of crop growth, with the concurrent with the depletion of hydrolysis products of urea in the system. However, in the present investigation, enhancement in available N content was observed in the presence of *Anabaena variabilis* under 120 kg N ha^{-1}. Vlek and Craswell (1979) reported a net immobilization of 18-30% of N from urea fertilizer by growing algal cells within 3 weeks of fertilizer application. Possibly, prevention of loss is due to immobilization by inoculated organisms and the release of immobilized N in turn may lead to reverse trends in inorganic N accumulation in soils under the urea N applied conditions .

Influence of BGA biofertilizer strains on plant parameters

There was a significant contribution through blue green algal inoculation on plant dry matter production measured during midcrop and harvest stage and this attribute was highest with *Anabaena variabilis* inoculation under natural as well as Phytotron conditions. The effect of blue-green algae on crop yield has also been attributed to the production of growth promoting substances by these organisms (Brown *et al.,* 1956; Kopteva, 1970; Tupik, 1973). Enhancement in rice seed germination, root - shoot growth, weight of rice grains and their protein content has been reported earlier (Shukla and Gupta, 1967; Venkataraman and Neelakantan, 1967; Singh and Trehan, 1973; Jacq and Roger, 1977) while others found a similar stimulatory effect on wheat (Gupta *et al.,* 1967), tomato (Kaushik and Venkataraman, 1979; Rodgers *et al.,* 1979),

radish (Rodgers *et al.*, 1979; Vorontsova *et al.*, 1988), peas (Gupta and Gupta, 1972), banana (Ganapathi *et al.*, 1994). Some researchers have described them as hormones *i.e.* cytokinin or auxin like substances (Rodgers *et al.*, 1979; Ahmed and Winter, 1968) or abscisic acids (Marsalek *et al.*, 1992) while others have described them as Vitamin B (Grieco and Desrochers, 1978) or amino acids (Watanabe 1951; Vorontsova *et al.*, 1988), antibiotics or toxins (Metting and Pyne, 1986). The production of these substances is, however, influenced by different stress factors (Marsalek *et al.*, 1992) as well as the application of chemicals, particularly cobalt salts (Venkataraman and Neelakantan, 1967). Certain mutants of BGA are known to produce more of these substances than their wild type counterparts (Vorontsova *et al.*, 1988).

In our investigation, blue green algal inoculation increased C content and N uptake in rice crop under Phytotron and natural conditions. These parameters were highest when pots were inoculated with *Anabaena variabilis* and significantly lower under uninoculated control conditions (Tables 29, 30.1, 30.2). The high carbon content reflects the productivity status of the plants, which in turn may be the effect of inoculation with blue-green algae.

N uptake by shoot as well as root exhibited an enhancement with the application of blue green algal strains and nitrogenous fertilizer under natural as well as Phytotron conditions. With the application of biofertilizer, N uptake has been shown to be more or less similar with the N uptake data at half dose of nitrogenous

Table 29. Influence of blue green algal inoculation on C content (g pot^{-1}) in plant during harvest stage

Strains	Phytotron conditions			Natural conditions		
	Shoot	Root	Total	Shoot	Root	Total
S_0	1.56	0.51	2.07	6.71	2.50	9.21
S_1	**2.70**	**0.94**	**3.64**	**10.09**	**4.37**	**14.46**
S_2	1.75	0.65	2.40	7.68	3.01	10.69
S_3	2.11	0.75	2.86	9.73	3.65	13.38
S_4	1.74	0.61	2.35	8.05	2.94	10.99

Table 30. Influence of blue-green algal inoculation on N uptake (mg pot^{-1}) by plant during harvest stage

30.1 Phytotron conditions

Strains	Shoot	Root	Grains	Total	% contribution by grains to total N
S_0	30.91	9.14	347.23	387.28	89.7
S_1	**59.53**	**18.02**	**530.19**	**607.74**	87.2
S_2	40.51	11.62	422.11	474.24	89.0
S_3	47.00	14.07	445.91	496.91	89.7
S_4	37.41	10.91	454.04	502.36	**90.4**

30.2 Natural conditions

Strains	Shoot	Root	Grains	Total	% contribution by grains to total N
S_0	66.8	9.27	502.96	579.03	86.9
S_1	**94.4**	**15.9**	**851.00**	**961.3**	88.5
S_2	70.8	10.5	559.88	641.18	87.3
S_3	85.4	12.2	630.56	728.16	86.5
S_4	66.6	10.9	691.95	769.45	**89.9**

S_0 = Without inoculation; S_1 = *Anabaena variabilis*; S_2 = *Aulosira fertilissima*; S_3 = *Nostoc muscorum*
S_4 = *Tolypothrix tenuis*

fertilizer, and *Anabaena variabilis* appears to be performing better in comparison to other biofertilizer strains for this attribute. The N recovered in the rice crop at maturity was lower than midcrop stage, which is in agreement with other reports (Norman *et al.*, 1992; Wilson *et al.*, 1989; Fernandez-Valientine *et al.*, 2000). These results suggest that the plants depend on native soil nitrogen to meet their N requirement during the later phases of vegetative growth (Kundu and Ladha, 1995; Wilson *et al.*, 1989). N uptake was 3 times higher under natural conditions when the pots were inoculated with *Anabaena variabilis*. However, under Phytotron conditions with *Anabaena variabilis* inoculation, the highest N uptake was about 1.5 times than that of control uninoculated conditions during both midcrop and harvest stage of rice crop.

N uptake by grains was enhanced through blue green algal application under natural and under Phytotron conditions indicating a fast mineralization of organic nitrogen in the soil accompanied by a rapid transfer of fixed N to the rice plants. The contribution of grain percentage N to the total N was 88.5% under natural conditions and 87.2% under Phytotron conditions. It has also been seen that the percent contribution by grains to the total N by plants was about 90% in the treatments wherein inoculation with *Tolypothrix tenuis* was done. Also, the soil/peat used in the present investigation for growing rice crop provided a favourable environment for the growth, establishment and proliferation of *Anabaena variabilis*. However, other researchers have reported much lower amounts of cyanobacterial nitrogen being recovered in

the plants (Fernandez-Valientine *et al.*, 2000; Tirol *et al.*, 1982). Direct evidence of transfer of cyanobacterial nitrogen to rice plant are however, scarce (Mac Rae and Castro, 1967; Roger, 1996).

Application of blue green algal biofertilizer enhanced the yield in comparison to control (uninoculated) conditions. Grain yield and its attributes were recorded to be highest with *Anabaena variabilis* inoculation as compared to other blue green algal strains tested. Both under natural as well as under Phytotron conditions, the grain yield with the application of BGA strains was shown to be *at par* with the grain yield observed at 50% of total recommended nitrogenous fertilizer application. Thus, it is clearly evident that the blue green algal application can result in the saving of substantial amounts of nitrogenous fertilizer for the marginal farmers in our Indian conditions. Similar results regarding influence of BGA on grain yield have been reported earlier (Sprent and Sprent, 1990; Yanni, 1992) and have been attributed to the favourable conditions for biological nitrogen fixation by blue green algae is under flooded conditions of rice fields.

Thousand grain weight remained more or less constant indicating that grain size is not affected by BGA inoculation. Sometimes, it is possible that under poor conditions, the plant-soil systems with organic fertilizers may be able to compensate towards the negative effects of poor environmental conditions and may even yield higher than the mineral fertilizer treatments. The possible compensation mechanisms may include increase in root growth and proliferation, improved nutrient uptake and translocation, modified

root-shoot ratio or improvement in other parameters such as photosynthetic efficiency or N-use efficiency.

When correlation coefficient (r) was analysed, it was found that available nitrogen and soil microbial biomass carbon correlated positively even at 1% probability with N uptake and C content in plants. The correlation between soil organic carbon with plant parameters however, was non-significant at both the stages of crop growth (Table 25).

Studies have indicated a significant correlation of soil organic carbon with total nitrogen and pH. However, other workers have reported that microbial biomass carbon and its seasonal changes were not correlated with either pH or total N content (Piao *et al.*, 2000a). Insam *et al.* (1991) also suggested that N availability may have little effect on microbial biomass carbon, although, their results have shown relationship between microbial biomass carbon and total N of the soil. Such analyses could not be undertaken in the present investigation.

Inoculation of soil with blue green algal strains improves N fertility of soil, rice yield and N uptake by the crop indicating that a large proportion of increased available N may be utilized by the rice crop (Roger and Reynaud, 1982). However, it has been reported that only one third of field population of blue green algae was decomposed and absorbed by the rice crop in the first year and the rest remained as residual soil nitrogen (Saha and Mandal, 1980). But, most of the residual nitrogen from BGA remaining in the soil after the harvest of crop does not persist after air drying of the soil.

Inoculation of rice fields with BGA may help to regenerate or improve soil structure because these organisms are known to excrete extracellularly a number of compounds like polysaccharides, peptides, lipids *etc.* during their growth which may possibly diffuse out and glue the soil particles together (Marathe, 1972; Bertocchi, 1990). Water stable aggregates which are an integral part of aggregate formation have been shown to increase significantly due to algal inoculation resulting in an improvement in the water holding capacity and aeration status of the soil (Subhashini and Kaushik, 1981; Roychoudhury *et al.*, 1983; Tiwari *et al.*, 1991). Such improvement in the soil structure due to algal inoculation may also favour better seedling emergence of the crops (Rogers and Burns, 1994) The effects of BGA in regeneration of physical properties of puddled soil have a special significance, where rice crop is followed by an upland crop like maize or potato, which need good tilth. However, as mentioned earlier, perceptible charges in the physical properties of soil may be achieved only from the implementation of long-term algalization practices. Additionally, BGA are also known to produce a large variety of secondary metabolites, particularly antibiotics and biotoxins (Frankmolle *et al.*, 1992; Kulik, 1995) which may act as growth deterrents to many unwanted organisms in soil.

Our investigation has clearly indicated that a reasonable blue green algae in combination with inorganic nitrogen fertilization can allow a significant reduction in N fertilizer input, without any significant loss in terms of productivity, and more importantly,

supplemented with the ecological benefits for the ecosystem. To achieve maximum benefits through use of phototrophic inoculum, the newly formed soil aggregates formed due to blue green algal inoculation must be left undisturbed. Inoculation may be required before each growing season and minimum tillage practices also need to be applied (Morgan, 1992) and inclusion of such approaches may help to maximize blue green algal nitrogen fixation, release of nitrogen to the environment and their contribution to C levels. The longer the period for which the soil is kept moist in the preflood period, the greater is likely to be the blue green algal standing crop. Addition of a suitable algal inoculum at an early stage of crop growth might lead to an extensive cover, which could persist over a greater period of time. The implication of relationships between BGA and other microbial populations in rice fields and their influence on the biochemical changes in the soil environment need to be further analysed in depth and observations have shown that BGA- soil microflora interaction can go a long way in C and N increment. Basic research into the biological aspects and *in situ* studies in rice fields need to be also undertaken for making BGA inoculation programs in rice fields more effective, viable and sustainable, particularly in relation to carbon and nitrogen contribution. So that they form a irreplaceable component of integrated nutrient management practices in rice based agroecosystems.

Summary

6. SUMMARY

The present investigation was aimed to analyze and evaluate the performance of blue-green algal biofertilizer strains from National Centre for Conservation and Utilisation of Blue Green Algae (NCCUBGA), IARI on certain specific soil properties in relation to carbon and nitrogen contribution and their effect on rice productivity. Morphological and cultural studies authenticated the identification of BGA biofertilizer genera as *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum* and *Tolypothrix tenuis*. Preliminary studies involved growth measurements (dry weight, specific growth rate and generation time), pigment production (chlorophyll, carotenoids and phycobilins), cellular constituents (soluble proteins and total sugars) and physiological attributes (nitrogenase, nitrate reductase, glutamine synthetase activity and ammonia excretion) under laboratory conditions. For each analysis, two per cent inoculum level was used as optimal rate of inoculation and experiments were conducted till late log phase in nitrogen free BG-11 medium.

Intergeneric variations were observed with respect to growth potential, pigment profile, nitrogen fixing ability and N assimilation related parameters in the blue green algal strains during incubation. These parameters revealed a typical sigmoid curve showing peak during the exponential active growth phase. Soluble proteins and total sugars were highest at 14^{th} and 21^{st} day of incubation while the N assimilation related parameters namely nitrogenase activity, glutamine synthetase enzyme and extracellular ammonia

release were higher at 14th day of incubation followed by a slow and gradual decline. Comparative performances of the strains indicated that parameters like chlorophyll, soluble proteins, total sugars and nitrate reductase activity were highest in *Aulosira fertilissima* and higher dry weight content was observed in *Anabaena variabilis*. *Nostoc muscorum* exhibited peak values for parameters like phycocyanin, allophycocyanin, total phycobiliproteins, nitrogenase activity on chlorophyll and protein basis and GS activity. Carotenoids and phycoerythrin were maximum in *Tolypothrix tenuis*.

Under pot culture conditions, rice crop was raised in peat under Phytotron conditions and in soil under natural conditions during *kharif* 2002. Blue green algal biofertilizer strains were taken alone or in combination with 3 levels of nitrogenous fertilizer and the results were compared with control uninoculated, -N and +N treatments in rice variety Basmati-1.

Under Phytotron conditions, the highest algal population was recorded with the application of *Anabaena variabilis* in the presence of 100% N given as Hoagland's solution. Under natural conditions, during midcrop stage, total algal population ranged from 11.36 under uninoculated condition to 15.49 CFU x 10^3 g^{-1} in treatment involving *Anabaena variabilis* inoculation. At harvest stage, the highest algal population (5.08 x 10^3 CFU g^{-1}) was observed in the pots inoculated with *Tolypothrix tenuis*.

The soil bacterial, fungal and actinomycetes population exhibited an enhancement with the application of nitrogenous fertilizer and was further

enhanced when BGA inoculum was provided. Bacterial population was maximum during midcrop as well as during harvest, when soil was inoculated with *Anabaena variabilis*. Fungal population analysed was highest in the pots inoculated with *Tolypothrix tenuis* during midcrop stage. However, during harvest stage, the highest fungal population was recorded when the pots received *Nostoc muscorum* as the inoculum. During midcrop stage, the highest actinomycetes population was recorded in presence of *Nostoc muscorum* and during harvest, *Anabaena variabilis* inoculation resulted in highest actinomycetes population. The data analysed has clearly indicated that the bacterial population contributed significantly to the total microbial flora. This revealed that even if artificial inoculation in the form of blue green algal application is given, maximum contribution to total microbial flora was through bacterial population only.

Studies have clearly indicated that the inoculation of soil with blue green algae can bring about appreciable changes in some of the electrochemical and chemical properties of soil particularly in relation to redox potential, availability of nitrogen and other nutrients, which might influence growth, and productivity of rice crops. Specific soil parameters like pH and EC were not influenced markedly due to blue green algal inoculation. However, pots inoculated with *Nostoc muscorum* showed highest redox potential during midcrop whereas, during harvest, *Anabaena variabilis* inoculation showed highest value.

At midcrop stage, the highest chlorophyll and dehydrogenase activity in soil samples were observed with *Tolypothrix tenuis* inoculation under 120

kg N ha^{-1}. During harvest, the soil chlorophyll and dehydrogenase activity were highest, when pots were inoculated with *Anabaena variabilis* at similar levels of N fertilizer. Highest nitrogenase activity was recorded when soils received *Nostoc muscorum* as inoculum under 60 kg N ha^{-1} during midcrop. During harvest, however *Anabaena variabilis* inoculation recorded maximum nitrogenase activity.

Microbial biomass carbon was enhanced with the application of blue green algal strains (in comparison to control conditions) with maximum values being recorded in treatment involving *Anabaena variabilis* inoculation during midcrop as well as during harvest. Microbial biomass carbon was maximum with *Tolypothrix tenuis* and *Anabaena variabilis* inoculation at 120 kg N ha^{-1}.

There was an enhancement in organic C content with the application of BGA biofertilizer strains and values were highest in pots inoculated with *Anabaena variabilis*. During midcrop stage, however, the highest values were recorded when pots received the application of *Nostoc muscorum*. It must be emphasized that there was an overall notable enhancement in this parameter during harvest in comparison to midcrop stage of crop growth. Organic carbon was highest during midcrop and harvest stage at 120 kg N ha^{-1} in the presence of *Nostoc muscorum* and at 60 kg N ha^{-1} in the presence of *Anabaena variabilis* application.

In the present investigation, available nitrogen increased with the application of blue green algal biofertilizer strains and was highest during midcrop as well as harvest stage in treatments involving *Anabaena variabilis*

inoculation while lowest available nitrogen was recorded in uninoculated control pots.

There was a significant contribution of blue green algal inoculation on plant dry matter production and this attribute was highest when evaluated at both stages of crop growth under *Anabaena variabilis* inoculation, under both Phytotron and natural conditions. Blue green algal inoculation was observed to increase C content and N uptake in rice crop under Phytotron and natural conditions. These parameters were highest when pots were inoculated with *Anabaena variabilis* and significantly lower under uninoculated (control) conditions. The enhanced carbon content reflects the productivity status of the plants, as a result of inoculation with blue-green algae.

N uptake by shoot as well as root improved with the application of blue green algal strains and nitrogenous fertilizer under Phytotron as well as natural conditions. With the application of biofertilizer, N uptake has been shown to be more or less similar with the N uptake data at half dose of nitrogenous fertilizer, and *Anabaena variabilis* performed better in comparison to other biofertilizer strains.

N uptake was 3 times higher under natural conditions when pots were inoculated with *Anabaena variabilis*. However, under Phytotron conditions with *Anabaena variabilis* inoculation, the highest N uptake was about 1.5 times than that of control (uninoculated) conditions during midcrop and harvest stage of rice crop. The results clearly indicted that the soil/peat used in the present investigation for growing rice crop exhibited favourable conditions for the development, establishment and proliferation of *Anabaena*

variabilis. The grain percentage N contribution to the total N contribution was 88.5% with inoculation of *Anabaena variabilis* under natural conditions and 87.2% under Phytotron conditions. It was also been observed that the percent contribution by grains to the total N by plants was about 90% when soil was inoculated with *Tolypothrix tenuis*.

Application of blue green algal biofertilizer enhanced the yield in comparison to control (uninoculated) conditions. Grain yield and its attributes were recorded to be highest with *Anabaena variabilis* inoculation, as compared to inoculation with other blue green algal strains tested. However, 1000 grain weight remained more or less constant indicating that the grain size is not affected by BGA inoculation. Comparison of data obtained after inoculation with BGA and that from 50% recommended N application revealed statistically at par values.

Correlation coefficient (r), when analysed between soil parameters (available nitrogen, organic carbon and soil microbial biomass carbon) and plant parameters (N uptake and C content) during midcrop stage and harvest stage, revealed that available nitrogen and soil microbial biomass carbon correlated positively even at 1% probability with N uptake and C content in plants. Thus, it is clearly evident that the blue green algal application can result in the saving of a significant amount of nitrogenous fertilizer, which can be particularly beneficial to the marginal farmers in our Indian conditions.

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* Originals not seen

APPENDIX

1. BG-11 medium

(For isolation of BGA, Stanier *et al.*, 1971)

	g L^{-1}
K_2HPO_4	: 0.04
$MgSO_4$	: 0.075
$CaCl_2$.$2H_2O$	: 0.036
Citric acid	: 0.006
Ferric ammonium citrate	: 0.006
EDTA	: 0.001
Na_2CO_3	: 0.02
Trace Metal Mix	: 1 mL

The trace metal mixture A_5 solution (Arnon, 1938) consists of the following constituents in g L^{-1}.

H_3BO_3	: 2.86
$MnCl_2$.$4H_20$	: 1.81
$ZnSO_4$.$7H_2O$	: 0.222
Na_2MoO_4 .$2H_2O$	: 0.390
$CuSO_4$.$5H_2O$	: 0.079
$Co(NO_3)_2$.$6H_20$	: 0.049

2. Martin's Rose Bengal agar medium

(For isolation of soil fungi, Martin, 1950)

	g L^{-1}
Glucose	: 5.0
KH_2PO_4	: 1.0
Mg SO_4 .$7H_2O$	: 0.5
Rose Bengal	: 0.03
Agar	: 20
Streptomycin sulphate (0.03% solution)	: 10 mL

3. Soil extract agar medium

(For isolation of soil bacteria, Parkinson *et al.*, 1971)

	g L^{-1}
K_2HPO_4	: 0.25
Yeast extract	: 5.0
Agar	: 20
pH	: 6.8-7.0
Soil extract	: 1000 mL

Preparation of soil extract:

500 g soil is mixed with 1 litre tap water, steamed in an autoclave for 1 hr at 121°C. The suspension was filtered through Whatman No 5 and the filtered extract was made up to 1 litre with distilled water

4. Starch casein medium

(For isolation of soil actinomycetes, Kuster and Williams, 1964)

	g L^{-1}
Soluble starch	: 10
Vitamin free casein	: 0.3
KNO_3	: 2.0
NaCl	: 2.0
K_2HPO_4	: 2.0
$MgSO_4.7H_2O$	: 0.05
$CaCO_3$	: 0.02
$FeSO_4.7H_2O$	: 0.01
Agar	: 20
pH	: 7.0

5. Hoagland's solution (Hoagland, 1944)

	mg L^{-1}
$Ca(NO_3).\ 4H_20$	: 950
$(NH_4)H_2PO_4$	: 120
KNO_3	: 610
$MgSO_4.\ 7H_2O$	: 490
H_3BO_3	: 0.6
$MnCl_2.\ 4H_2O$	: 0.4
$ZnSO_4.\ 7H_2O$	: 0.09
$CuSO_4.\ 5H_2O$	: 0.05*
H_2MoO_4	: 0.02
$Co(NO_3)_2.\ 6H_2O$	: 0.025
$FeSO_4.\ 7H_2O$	: 24.8
NaOH	: 6.6
EDTA	: 33.2
pH	: 5.3-5.5

* In case of deficiency symptoms the dose should be doubled

6. Modified Hoagland's solution:

	mg L^{-1}
$NH_4\ NO_3$	: 503 (100% N)
$CaCl_2.\ 2H_2O$	: 802
KH_2PO_4	: 140
KCl	: 373
$MgSO_4.\ 7H_2O$	: 490
H_3BO_4	: 0.6
$MnCl_2.\ 4H_2O$	: 0.4
$ZnSO_4.\ 7H_2O$	: 0.09
$CuSO_4.\ 5H_2O$	: 0.05
H_2MoO_4	: 0.02
$Co(NO_3)_2.\ 6H_2O$	: 0.025
$FeSO_4$	: 24.8
NaOH	: 6.6
EDTA	: 33.2

ABBREVIATIONS

cm	:	centimetre
cm^{-3}	:	cubic centimeter
chl	:	chlorophyll
CFU g^{-1}	:	colony forming units/gram
CD (p=0.05)	:	critical difference at 5 per cent level
dS m^{-1}	:	deciSiemen / metre
°C	:	degree Centigrade
EC	:	Electrical Conductivity/ Enzyme code
FID	:	flame ionization detector
g	:	gram
ha	:	hectare
h	:	hour
h × d	:	height × diameter
i.d.	;	inner diameter
kg	:	kilogram
L/D	:	light/dark cycles
M-STATC	:	Michigan State University Statistical Analysis Package
μg	:	microgram
μl	:	microlitre
μm	:	micrometer
μmol	:	micromole
mg	:	milligram
mL	:	milliliter
mm	:	millimetre
mM	:	millimolar
min	:	minute
M	:	molar
nm	:	nanometer
nmol	:	nanomole
N	:	normal
O.D.	:	optical density
%	:	percent
TCA	:	trichloroaceticacid
SE(m)	:	standard error of mean
v/v	:	volume / volume

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