CHAPTER II

REVIEW OF LITERATURE

II.1 Morphology

The blue-green algae (cyanobacteria) are an unique group of prokaryotic photosynthetic microorganisms. They show morphological variations having unicellular, filamentous, multiseriate, unbranched and branched filamentous forms. The cells of unicellular algae are usually spherical or cylindrical which remain separately within a well defined mucilaginous sheath. In the colonial form, the cells remain aggregated after cell division to form distinct colonies of specific forms. The filamentous form constitutes many cells under one plane beneath a sheath. A typical blue-green algal cell under light microscope shows a definite shape, surrounded by a firm cell wall which is enclosed by a mucilaginous sheath. The alga inside the filamentous sheath is a trichome. Thick hyaline walls of rounded or cylindrical shape are heterocysts positioned either terminal or intercalary. Filamentous heterocystous blue-green algae also show true or false branching. Spores, the perenating structures which
usually larger than vegetative cells are spherical or cylindrical in shape (Fogg et al., 1973).

II. 2 Distribution

2.1 General

Blue-green algae are widely distributed in a wide variety of habitats. BGA are known to grow aerobically, micro-aerobically and anaerobically, exhibiting photosynthetic, heterotrophic and photoheterotrophic nutrition. They are reported to withstand extremes of temperature and desiccation (Tripathy and Talpasayi, 1980). Their distribution is well documented.

BGA occur either as free-living or as a symbiont in temperate regions as reported in Russia (Shtina, 1969), Northern and Western Europe (Henriksson, 1971; Henriksson et al., 1972; Granhall, 1975), North America (Shields and Durrell, 1964; Mayland et al., 1966; Jurgensen and Davey, 1968), Polar regions (Alexander, 1975) and fresh water habitats (Fogg, 1971; Renaut et al., 1975). The ecology of the BGA is something of a paradox because these organisms are of worldwide distribution being reported from the Antarctic to the tropics, however, abundantly occur in localised situations such as rice paddy soils.
2.2 Distribution in rice fields

Available literature indicated the ubiquitous distribution of blue-green algae in the rice fields. Reports of different parts of the world including Egypt (El-Nawawy and Hamdi, 1975); Morocco (Renauds and Sasjian, 1970); Mali (Trarore et al., 1978); Spain (Batalla, 1975); Italy (Ciferri, 1963); Hungary (Kol, 1956); Russia (Prikhod’kava, 1971); Australia (Bunt, 1961); California (Chapman et al., 1972); Cuba (Goryunova and Orleanskii, 1970); South and South-east Asia (Watanabe, 1959a); Iraq (Al-kaisi, 1976); Malaysia (Johnson, 1969); Java (Jutono, 1973); Japan (Okuda and Yamaguchi, 1952a, 1956a, b; Konishi and Seino, 1961); Philippines (Fantastico, 1977) and Sri Lanka (Kulasooriya and De Silva, 1978).

Likewise, the occurrence of BGA from different parts of India has been extensively studied by several workers (Singh, 1939, 1942, 1961; Gonzales and Gangla, 1949; Mitra, 1951; Pandey, 1965; Amma et al., 1966; Patnaik, 1966; Shukla, 1971; Tiwari, 1972). The existence of several nitrogen-fixing BGA in C.R.R.I., Cuttack experimental farm plots have been recorded (Singh, 1961; Subrahmanyan et al., 1965c). However, Singh (1973b, 1978a) documented the abundance of
Anaphanothece sp., Anabaena sp., Aulosira sp., Cylindrospermum sp., Gloeotrichia sp. and Nostoc sp. Interestingly, Venkataraman (1975) observed the regional distribution of dominant forms like Aulosira fertilissima, Nostoc sp., Anabaena sp. in Indian states. Their dominance differed from place to place.

II.3 Nitrogen fixation

Nitrogen fixation is mediated by the enzyme nitrogenase. The available information has established that BGA contribute nitrogen substantially which are of tremendous importance in tropical agriculture.

Frank (1889) obtained evidence for nitrogen fixation in soils containing blue-green algae. Schloesing and Laurent (1892) demonstrated nitrogen fixation in pure cultures of heterocystous Nostoc punctiforme and Nostoc minutum, whereas the non-heterocystous Microcoleus vaginatus did not. However, these findings remained controversial for a long period. Later, Drewes (1928) showed that pure culture of Anabaena variabilis, Anabaena sp., Nostoc punctiforme grew well in a medium free of combined nitrogen. Allison et al. (1937) further confirmed nitrogen fixation by Nostoc muscorum. De (1939) demonstrated
nitrogen fixation by Anabaena gelatinosa, *A. nivaculoides* and *A. variabilis* isolated from rice fields of Faridpur (now in Bangladesh) and while Singh (1942) further confirmed the contribution of *Aulosira fertilissima*, *Anabaena ambigua*, *A. fertilissima*, *Cylindrospermum gorakhporense* from Indian paddy soils which led to the recognition of these organisms in nitrogen economy of paddy soils. Singh reported nitrogen fixation by unicellular blue-green alga *Aphanathece* sp. (1973c, 1977).

Presently, more than 125 strains of blue-green algae are known to fix nitrogen (Stewart *et al.*, 1979). Based on the morphology and conditions of functional nitrogenase *in vivo* Stewart *et al.* (1979) broadly distinguished blue-green algae into four major groups. Firstly, that the heterocystous forms capable of fixing $N_2$-aerobically, microaerobically and anaerobically. Secondly, that non-heterocystous unicellular forms with nitrogenase activity under aerobic conditions. The third group includes pleurocapsalean forms reducing acetylene anaerobically. The fourth group of non-heterocystous filamentous forms capable of nitrogenase activity under microaerobic or anaerobic conditions. Thus, a large variety of blue-green algae which were hitherto unknown as $N_2$-fixers has been reported as nitrogen fixers.
3.1 Methodology

The rate of nitrogen fixation can be determined with the help of any one of the methods, listed below in order of increasing sensitivity.

3.1.1 Increase in total nitrogen as determined by Kjeldahl analysis (Shiori et al., 1944; Okuda and Yamaguchi, 1955; Singh, 1961; Singh, 1976).

3.1.2 Measuring the change in nitrogen:Argon ratio (Koyama, 1966; Wada et al., 1978).

3.1.3 Incorporation of elemental nitrogen enriched with $^{15}\text{N}_2$ into algal protein (MacRae and Castro, 1967; Burris, 1974).

3.1.4 Reduction of acetylene to ethylene (Hardy et al., 1973; Lee and Watanabe, 1977; Matsuguchi, 1978).

3.2 Algal biomass estimation

Only few reports are available on biomass determination of BGA in submerged rice fields perhaps due to the problems of methodology in estimating the algal growth qualitatively as well as quantitatively.
3.2.1 Dilution techniques and plating

This method is advantageous since it provides qualitative and quantitative results depending on dilution reliability. However, Peterson (1932) noticed that filamentous forms are difficult to be separated into individual cells while Pandey (1965) observed that filamentous forms are easily separated which might give inflated figures of abundance. Roger and Reynaud (1976) improved the methodology by determining the mean volume of each "Count unit" by directly examining the first dilution and multiplying the results by its "volume unit". However, it is tedious and it cannot distinguish between active and inactive forms.

3.2.2 Pigment analysis

Acetone dissolved pigments also give the total algal biomass and mycoxanthin present in BGA biomass (Singh, 1961). However, this method is not free from defect as ascribed that humic acid interferes during extraction (Fogg et al., 1973).

3.2.3 Direct microscopic examination

Qualitative studies under ordinary or fluorescence
microscope have been performed (Tchan, 1953; Drew and Anderson, 1977). This method was also used for quantitative analysis by Saito and Watanabe (1978).

3.2.4 Direct weighing method

Roger and Kulasooriya (1980) suggested algal biomass measurement by weighing directly if the quantity is sufficient for weighing. This method was found to be better to avoid tedious, imprecise and ambiguous measurements. Singh (1979) and Kulasooriya et al. (1980) depicted biomass of the fresh form by weight. Since water content of the algal body varies largely, Roger and Kulasooriya (1980) cautioned the expression on wet weight. Possibly, representation of biomass on dry weight basis seemed reliable (Singh, 1976, 1979; Saha et al., 1982).

II.4 Inoculum production

Despite the voluminous literature on the beneficial effect of blue-green algae, information on the mass production of BGA is limited. However, mass culture technologies have been reviewed by several workers (Venkataraman, 1969, 1972; Watanabe, 1975).
4.1 Selection of strong nitrogen fixers

Stewart et al. (1979) reviewed the importance of selection of the good nitrogen-fixing potential strains which were summarised as follows:

i. that could grow very rapidly as Nostoc sp. having 5-6 hr of generation time

ii. that could fix nitrogen equally under aerobic, microaerobic and anaerobic conditions in order to tolerate a wide range of oxygen tension found in rice fields (heterocystous and some unicellular forms)

iii. that could fix nitrogen under photo-autotrophic, photo-heterotrophic chemoheterotrophic conditions (for example, Anabaena sp., Anabaenopsis sp., Chlorella sp., Nostoc sp., Tolypothrix sp.)

iv. that could evolve less hydrogen for avoiding wastage of ATP in the metabolic activities (as Anabaena cylindrica)
v. that could withstand high nitrogenous compounds found in the paddy fields

vi. that could liberate substantial amount of readily assimilable extra cellular nitrogen.

Grant and Alexander (1981) also suggested for using the inocula of algae which are not suitable for active grazing by the predators. For laboratory studies mainly the single strain cultures like *Aulosira fertilissima*, *Tolypothrix tenuis*, *Nostoc* sp. have been used (Stewart *et al.*, 1979). For field studies single as well as multi-strained cultures have been used. The use of a mixture of several BGA cultures has been recommended by several workers (Subrahmanyan, 1972; Venkataraman, 1972).

Ley *et al.* (1959) selected the alga *Anabaena azotica*, *A. variabilis* in China.

In Japan, Watanabe (1959a) screened three good *N₂*-fixing algae out of sixteen species. Again out of that *Tolypothrix tenuis* and *Calothrix brevissima* had higher activity, whereas *Anabaenopsis circularis* possessed low activity. Watanabe (1962) further confirmed
Tolypothrix tenuis as superior to Calothrix brevissima in N₂-fixing ability.

In India, Singh (1961), Sundara Rao et al. (1963) used the alga Aulosira fertilissima. Subrahmanyan et al. (1965c) used mixed cultures of Nostoc sphaericum, N. amplissum, Tolypothrix campylonemoide, Westiella sp. Sankaram et al. (1967) also used mixed cultures of Nostoc, Anabaena, Scytonema, Venkataraman and Goyal (1969a, b), Goyal and Venkataraman (1970) used the selected mixture of Aulosira fertilissima, Tolypothrix tenuis, Cylindrospermum muscicola, Nostoc sp. Singh (1976) used Aulosira sp., Singh (1978) reviewed the importance of Aulosira sp., Cylindrospermum sp., Nostoc sp., Anabaena sp., Gloeotrichia sp., Anphanathece sp. for various fundamental as well as applied research. It is recommended in All India Coordinated Project on Algae (1979) to inoculate mixed cultures of Aulosira sp., Tolypothrix sp., Scytonema sp., Nostoc sp., Anabaena sp. and Plectonema sp.

4.1.2 Quantity of inoculum

Literature showed much variation in the quantity of inoculum used in the field experiments either for
seeding purposes or for proliferating in the paddy field for providing beneficial effect to the paddy. The documented quantity in terms of dry weight/ha is as follows: 200 g (Subrahmanyan et al., 1965b; 5 g (Sankaram et al., 1967); 1 kg (Venkataraman and Goyal, 1969). However, Venkataraman (1972) recommended the use of 1-6 kg/ha of inoculum with paddy. Nevertheless, Venkataraman (1977) further suggested that excess inoculum helped better algal production and establishment.

4.1.3 Form of inoculum

Singh (1961) adopted dry inoculum containing spores to the puddled fields. Venkataraman (1972, 1977) recommended the dried soil based culture. On the other hand, Jha et al. (1965) applied fresh form of blue-green algae. Sankaram et al. (1967) and Sankaram (1971) used fresh form of inoculum. Subrahmanyan and Manna (1966) observed that the growth of inoculum in the fresh form was vigorous against the dry inoculum. Alimagno and Yoshida (1975) compared both forms like dried and fresh inoculum on paddy and indicated the superiority of the fresh form over the dry inoculum.
4.1.4 Methods of inoculation

Reviews of Sankaram (1971), Venkataraman (1972) depicted much variation in methodology. Singh (1961) adopted broadcast method by mixing the algae powdered with lime on the surface of puddled soil prior to transplantation. Jha et al. (1965) mixed the fresh inoculum with the soil which was inoculated later. Sankaram et al. (1967) transferred the inoculum to the washed river sand and the sand culture was uniformly spread, a week after transplanting. The comparative efficiency of four methods of algal inoculation was inconclusive (Sankaram, 1971). Venkataraman (1972) mixed the multi-strain soil based culture with a bucket of water containing molybdenum (0.5 kg sodium molybdate/ha) and sprinkled over the rice field water one week after transplantation. In direct sown paddy, the culture is mixed with rice seeds (Venkataraman, 1972).

4.2 Mass scale inoculum production

Two ways of algal production like production under artificial conditions and in field are distinguished.
4.2.1 Production under artificial conditions

4.2.1.1 Tank culture

Specially designed tanks having arrangement for the culture with glass windows through which the culture is illuminated from outside are used (Watanabe, 1959b).

4.2.1.2 Closed circulation culture

Watanabe (1959b) used a flat tube using vinyl sheeting for algal production.

4.2.1.3 Gravel culture

Culture on the moist surface developed by Watanabe (1959c) on volcanic gravel which was later transferred to field inoculation for algal proliferation. Venkataraman (1972) grew the inoculum on synthetic sponge which was reported growing luxuriantly.

4.2.1.4 Open bubbling system

Watanabe (1959b) used hot spring water as source of heat and natural gas as source of carbon dioxide. The air was bubbled constantly through the culture solution.
4.2.1.5 Continuous culture system

Continuous culture of filamentous blue-green algae used by Thomas (1973) showed rapid growth of algae with continuous addition of the medium and algal harvest at the similar rate. Mass cultivation of \textit{Anacystis nidulans} in long vertically arranged glass tubes was reported by Juttner (1973). Singh (1974a) cultured the algae using medium in the tray under green-house conditions where growth of \textit{Aulosira} sp. was better in the medium at higher temperature, but \textit{Anabaena} showed luxuriant growth in nitrogen free and nitrate containing medium.

4.3.1 Open air soil culture

Few reports are available on algal production by utilizing the natural sun light under simulating rice field so as to minimise the cost of production and at the same time maximising the algal out-put.

4.3.2 Mass culture in the fields

Venkataraman (1972) grew algae in galvanized iron trays with a layer of soil. By similar method, Singh (1979) also got encouraging results in the
trays. Misra (1979) suggested the production of blue-green algae in concrete tanks with soil.

BGA have also been produced under field conditions enclosed by levee (Venkataraman, 1968, 1972; Pantastico and Gonzales, 1976; Kananaian, 1979; Srinivasan, 1979). In China, blue-green algae were grown in the rice nursery bed or in the field between two crops (Academima Sinica, 1978). All India Coordinated Project on Algae (1979) recommended the production of algae in the galvanised iron trays or in brick and mortar structures using 8-10 kg soil, 200 g superphosphate, carbofuran 25 g and maintained at 2-6 depth. Srinivasan (1980a,b) cultivated blue-green algae in the field by using 5 kg of culture, 2 kg superphosphate, 250 g of carbofuran maintaining 5 cm of standing water in 40 metre square area. He further suggested BGA production in ponds and ditches. Venkataraman (1981) reported that the production of BGA is no longer a scientific concept but a infra-structure to the farmers. Some states in India like Tamil Nadu, Uttar Pradesh, Andhra Pradesh, Pondicherry, Madhya Pradesh, Maharashtra, Kerala, Karnataka, Jammu and Kashmir, Bihar, and West Bengal have established algal production centres to popularize large scale production of blue-green algae.
4.3.3 Rate of biomass production under laboratory conditions

Watanabe (1959b) obtained 0.2 g (dry weight/litre/day) by tank culture method. Similarly, 0.18 g/litre/day yield of BGA was observed by Venkataraman (1972). Watanabe (1959) recorded 7.9 g/dry weight/m²/day through closed circulation method, whereas Watanabe et al. (1959) recorded the growth rate of BGA equivalent to 6.4 g (dry weight)/m²/day which corresponds to 7 tonnes of alga per annum.

4.3.4 Rate of algal production in field conditions

Venkataraman (1972) obtained an average production of 20 g (dry weight)/m²/day employing air soil culture method. The rate of production of algal flakes ranged from 0.4 to 1 kg m² in 15 days (Misra, 1979; Kanaynaiyan, 1979; Srinivasan, 1979). However, Srinivasan (1980a) got yield of 16-35 kg dry weight/40 m² in the field plots. Srinivasan (1980b) further reported that the algae multiplied in ponds and ditches produced 10-40 kg/40 m².

4.4 Conditions for algal production in the paddy field

Blue-green algal production vary considerably due
to climatic as well as physico-chemical factors. Roger and Reynaud (1979) reported that BGA community was less affected by chemical properties of the soil, than by the climate and composition of flood water.

II.5 Climatic factors

5.1.1 Light

Autotrophic nitrogen-fixing microorganisms derive energy from solar radiation. Algae are restricted to the photic zone and are located in the upper layer of the soil or floor water. Vertical migration of algae also takes place in relation to \( O_2 \) production (Reynaud and Roger, 1978). Kurasawa (1956) investigated the relationship between the plant canopy and the availability of light to the algae and reported that 30 and 60 cm height of the rice plant suppressed 50 and 90 percent of light availability respectively which affected algal growth. Strong light intensity inhibited the growth and nitrogen-fixing capacity of blue-green algae (Brown and Richardson, 1968). Blue-green algae has been considered as sciophilous plant (Roger and Reynaud, 1979).

Inhibition of algal nitrogen fixation occurred at a light intensity 70 K lux (Reynaud and Roger, 1978).
Nitrogen fixation by *Gloeotrichia* sp. was saturated at 10 K lux light intensity and 5 K lux produced 87 percent value (IRRI, 1976). However, certain algae like *Cylindrospermum* sp. reported to be resistant to light intensity in the paddy field (Trarore et al., 1978). Field growth of inoculated *Aulosira fertilissima* in high sun-light was reported to grow better (Singh, 1976). Since algae have different light adaption abilities, light might have a selective effect on the composition of the blue-green algal flora (Roger and Kulasooriya, 1980).

5.1.2 Temperature

The optimal temperature for the growth of blue-green algae was about 35°C which was higher than eukaryotic algae (Sorokin, 1959; Fogg et al., 1973). Subrahmanyan (1972) at CRRI, Cuttack observed a set back of growth during cold seasons in pot and field conditions. Roger and Reynaud (1976) recorded inhibitory effect on BGA during dry season in temperate zones. On the other hand, Singh (1976) reported that high temperature of 34-39°C in paddy water was favourable for *Aulosira fertilissima* growth. Jones (1977) attributed algal inhibition to high temperature. However, •
temperature is rarely a limiting factor in paddy fields (Roger and Reynaud, 1979).

5.1.3 Rainfall

Heavy rains suppressed the development of *Aulosira fertilissima* inoculated in the field (Singh, 1976). Buffeting rains mix the algae with the soil ultimately disallow nitrogen-fixing ability and biomass (Trarore et al., 1978). Rainfall increases water turbidity which suppresses the phototrophic nitrogen-fixing ability. By comparing the ARA, before and after typhoon, Roger and Kulasooriya (1980) observed drastic reduction in ARA.

5.2 Biotic factors

5.2.1 Algal grazers

Grazers are the major disturbing factors for the establishment of the BGA (Hirano et al., 1955; Watanabe et al., 1955). Invertebrates like cladocerans, copepods, ostracods, mosquito larvae, snails are common grazers of algae in rice fields (Roger and Kulasooriya, 1980). Kurasawa (1956) observed that zooplankton appeared after 1 week of phytoplankton growth and attained
 maximal biomass after 2 weeks. Watanabe et al. (1959) depicted preferential grazing that unicellular green algae are excellent feed for daphnids while filamentous \( \text{N}_2 \)-fixing algae served as nutrient source.

The fresh water fish, \textit{Tilapia mozambica} fed on the algal grazers like Chironomidal larvae, which is indirectly showed a beneficial effect on \textit{Nostoc commune} (Marathe, 1964). However, \textit{Tilapia nilotica} has been known to ingest large quantity of BGA (Roger and Reynaud, 1979). Frequent failure of inocula of algae to enhance algal production has been documented.

Wilson et al. (1980a) presented evidence from laboratory experiments that ostracods, when present in large numbers prevented the development of \textit{Tolypothrix tenuis} and Osa-Afiana and Alexander (1981) reported a reduction in nitrogen fixation by \textit{Tolypothrix tenuis} and \textit{Anabaena} sp. Established populations of \textit{Aulosira} sp., \textit{Calothrix} sp., \textit{T. tenuis} were more resistant to predation by \textit{Cypris} sp. Grabbour et al. (1980) reported protozoa and nematodes as algal growth retarders.

Grant and Alexander (1981) observed grazing of five sps. of algae by an ostracod, \textit{Cypris} sp. Animal size and flood water temperature affected the feeding of
Ostracods. Use of lindane controlled the insect population.

5.3 Physico-chemical factors

5.3.1 pH

pH is one of the main factors controlling the occurrence and growth of blue-green algae. In natural conditions, it grow in environment that are neutral to alkaline, a few species are reported to grow in a pH 5.0-6.0 (Fogg, 1956). *Aulosira fertilissima* and *Calothrix brevissima* grew in Kerala rice fields having pH 3.5-6.5 (Aiyer, 1965). Heavy growth of BGA was observed in an acidic soil with a pH of 5.5 due to surface application of straw (Roger and Kulasooriya, 1980).

A positive correlation between water pH and the abundance of blue-green algae is reported. Higher pH in supernatant water than the soil was observed by Okuda and Yamaguchi (1956b). Granhall and Henriksson (1969) correlated between the occurrence of BGA and soil pH. Similarly growth of *Aphanothece* sp. at different soil pH was observed (Singh, 1974b, 1978a). García et al. (1973) demonstrated significant
correlation between pH of paddy soils (4.0-6.8) and abundance of \( \text{N}_2 \)-fixing BGA in the soils. Similarly, Wilson and Alexander (1979) observed positive response of soil pH on growth of BGA. Reynaud and Roger (1978) presented positive correlation between soil pH and the \( \text{N}_2 \)-fixing biomass.

5.3.2 Phosphorus

Phosphorus is one of the major nutrients required for the growth and nitrogen fixation of blue-green algae. BGA assimilate more phosphorus than its vital need. They store the excess amount in form of poly-phosphate for the future use (Butterton and Van Baalen, 1968).

In the laboratory conditions, Stewart et al. (1970) established that P deficient cultures had low ARA and addition of phosphorus induced stimulation of nitrogenase activity within 15-30 min. The response to phosphorus was bioassayed (Stewart and Alexander, 1971) and good response was evident from the growth of BGA with the application of phosphorus (Wilson and Alexander, 1979). Under field condition, BGA growth was closely correlated with the available P content.
of the soil. The growth was low at 0-5 ppm but high above 6 ppm (Okuda and Yamaguchi, 1952b). Application of phosphate fertilizer to the paddy field is favourable to the growth and nitrogen fixation of the inoculated and native algae (Shioiri et al., 1944; De and Biswas, 1952; De and Mandal, 1956; Arora, 1969; Than, 1969).

5.3.3 Nitrogen

In natural conditions, nitrogen is not the primary nutrient which limits the growth of N₂-fixing algae. The principal sources of nitrogen correspond to the main forms of N fertilizers used in rice cultivation. Scanty information is available on the competition between N₂-fixing and non-N₂-fixing forms affected by the nature and the concentration of the synthetic nitrogen. Laboratory experiments revealed the inhibitory effect of combined N on growth and N₂-fixation of BGA (Patnaik and Singh, 1977; Singh, 1975; Singh, 1978b). However, the selective action and inhibitory effect of nitrogenous fertilizers on nitrogen-fixing ability have been explained. Anabaena sp. was observed only in unfertilized plots (Okuda and Yamaguchi, 1952a). Subrahmanyan (1965c) noticed enhancement of growth of green-algae with N fertilizers but BGA growth was
reduced. Yoshida et al. (1973) reported that application of N fertilizer increased green algal growth, whereas growth of BGA was abundant in the absence of N fertilizer. $N_2$-fixation declined as much as 72 per cent by ammonium sulphate, 98 per cent by ammonium chloride at 200 kg N/ha and completely inhibited at 400 kg N/ha (Roger and Kulasooriya, 1980).

5.3.4 Potassium

Potassium when applied singly had no effect on the growth of BGA (De and Sulaiman, 1950; Marathe, 1963; Mahapatra et al., 1971). If potassium was applied with nitrogen and phosphorus it had little effect (Marathe, 1963). However, a depressive effect at higher dose was reported (Mahapatra, 1971).

5.3.5 Lime and molybdenum

Generally, liming is done to limit the fall in pH. The application of CaCO$_3$ has been found to encourage BGA growth and $N_2$-fixation (Okuda and Yamaguchi, 1952a,b; Nishigaki and Shioiri, 1959; Amma et al., 1966; Than, 1969; Yamaguchi, 1976). Subrahmanyan et al. (1965c) observed better growth of BGA due to application of sodium molybdate of
0.25 kg/ha. Molybdenum is also a limiting factor during active nitrogen-fixing state (Stewart et al., 1979). However, minimum level (0.2 ppm) of molybdenum is generally available in paddy soils (Roger and Kulasooriya, 1980).

5.3.6 Insecticides

Although use of insecticides is a common practice to control algal grazers and rice pests, little information is available with regard to their effect on BGA when applied under field conditions. The studies and recommendations in the laboratory cannot be taken as authentic for the field because the toxicity, rate of degradation are entirely different (Roger and Kulasooriya, 1980).

5.6.1 Inhibitory effect

Resistance to pesticides varies from strain to strain. *Cylindrospermum* sp. was found to be less resistant to insecticides than *Aulosira fertilissima* and *Plectonema boryanum* (Singh, 1973a). Similar inhibitory effect was reported (Das and Singh, 1978, 1979; Kar and Singh, 1978a,b). *Aulosira fertilissima* tolerated 4 insecticides at higher rates than
recommended levels (Ahmad and Venkataraman, 1973).

5.6.2 Stimulatory effect

Hirang et al. (1955) demonstrated that 1-5 ppm of parathin was fatal to algal grazers but in no way inhibitory to Tolypothrix tenuis. Raghu and MacRae (1967) attributed the development of heavy algal bloom due to the application of gamma-BHC to control the stem borer. Growth of Aulósira fertilissima was enhanced up to 10 ppm, recommended dose being 1-2 ppm (Ahmad and Venkataraman, 1973). Similarly, the stimulatory effect of carbofuran has also been observed (Kar and Singh, 1978a,b). Encouraging effect on algal growth was noticed using field dose of insecticides furadan (12.5 g/m²), BHC (15 g/m²), phorate (10 g/m²), carbofuran (6 g/m²) (Srinivasan et al., 1977).

II.6 Blue-green algae with rice

6.1 Succession of algae during cultivation cycle

Reynaud and Roger (1978) presented a model of algal succession in rice fields which is summarised as: (i) from planting to maximum tillering - diatoms.
and unicellular green algae, (ii) from tillering to panicle initiation - filamentous green algae and non-heterocystous BGA, (iii) heading - heterocystous and non-heterocystous BGA under dense plant canopy or filamentous and heterocystous BGA under thin plant canopy. Similar patterns were also reported by Pantastico and Suayan (1973) in Philippines and Kikuchi et al. (1975) in Japan. However, the occurrence of \( \text{N}_2 \)-fixing algal blooms in Mali at earliest stage of paddy growth was noticed (Trarore et al., 1978) and \( \text{N}_2 \)-fixing forms were found throughout the cultivation period in Sri Lanka (Thirukkaanasan et al., 1977).

6.2 Variation of algal biomass during cultivation cycle

Watanabe (1951) reported that the growth of blue-green algae was more in the presence of rice plants. Similar finding was presented by Watanabe et al. (1978b) and Kulasooriya et al. (1980a). The prevalence of blue-green algae largely varies from a few to \( 10^7 \) g dry soil as well as from few kg to tonnes of algae/ha in flooded rice fields. Tables 1 and 2 briefly the algal biomass in paddy fields (Roger and Kulasooriya, 1980). Development of algal biomass could occur at any time.
Table 1. References reporting algal enumerations in rice fields (cited from Roger and Kulasooriya, 1980)

<table>
<thead>
<tr>
<th>References</th>
<th>Location</th>
<th>Values (no./g dry soil)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araragi et al. (1978)</td>
<td>Thailand</td>
<td>10 to $10^6$</td>
<td>9 soil types studied</td>
</tr>
<tr>
<td>Araragi and Tangcham (1979)</td>
<td>Thailand</td>
<td>$10^3$ to $10^5$</td>
<td>103 sites studied</td>
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<tr>
<td>Garcia et al. (1973)</td>
<td>Senegal</td>
<td>0 to $10^6$</td>
<td>40 soils studied during the dry season</td>
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<tr>
<td>Ishizawa et al. (1975)</td>
<td>Japan</td>
<td>$10^6$</td>
<td>Fertilized plots</td>
</tr>
<tr>
<td>Kobayashi et al. (1967)</td>
<td>Thailand</td>
<td>$10^3$ to $10^5$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malaysia</td>
<td>$10^4$ to $10^7$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Philippines</td>
<td>$10^3$ to $10^5$</td>
<td></td>
</tr>
<tr>
<td>Matsuguchi et al. (1970),</td>
<td>Thailand</td>
<td>$10^2$</td>
<td>Brackish water, alluvial soil and Regosol</td>
</tr>
<tr>
<td>Matsuguchi and Tangcham (1974),</td>
<td></td>
<td>$10^3$</td>
<td>Noncalcic brown soil</td>
</tr>
<tr>
<td>Matsuguchi et al. (1974),</td>
<td></td>
<td>$10^4$</td>
<td>9 other soil types</td>
</tr>
<tr>
<td>Matsuguchi et al. (1976)</td>
<td></td>
<td>$10^5$</td>
<td></td>
</tr>
<tr>
<td>Traore et al. (1978)</td>
<td>Mali</td>
<td>10 to $10^6$</td>
<td>12 measurements in the same field along a 2-year period</td>
</tr>
<tr>
<td>Singh (1978a)</td>
<td>India</td>
<td>$2 \times 10^7$ cm$^2$</td>
<td>Aphanothece pallida from the water surface</td>
</tr>
</tbody>
</table>

*a* Most-probable-number method.  
*b* Plating method.  
*c* Method not indicated.
Table 2. References reporting algal biomass measurements in rice fields (cited from Roger and Kulasooriya, 1980)

<table>
<thead>
<tr>
<th>References</th>
<th>Location</th>
<th>Dry weight (kg/ha)</th>
<th>Fresh weight (kg/ha)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academia Sinica (1978)</td>
<td>China</td>
<td></td>
<td>7,500</td>
<td>After inoculation</td>
</tr>
<tr>
<td>Mahapatra et al. (1971)</td>
<td>India</td>
<td>3 to 300</td>
<td>60 to 6,000</td>
<td>Green algae dominant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>600</td>
<td>N₂-fixing BGA dominant</td>
</tr>
<tr>
<td>Muzafarov (1953)</td>
<td>UzbSSR</td>
<td></td>
<td>16,000</td>
<td>Total algal biomass</td>
</tr>
<tr>
<td>Renaud and Sasson (1970)</td>
<td>Senegal</td>
<td>2 to 6,000</td>
<td>2 to 2,000</td>
<td>Total algal biomass</td>
</tr>
<tr>
<td>Saito and Watanabe (1978)</td>
<td>Philippines</td>
<td>2 to 114</td>
<td></td>
<td>N₂-fixing algal biomass</td>
</tr>
<tr>
<td>Singh (1976)</td>
<td>India</td>
<td>480</td>
<td>9,000</td>
<td>Aulosira bloom</td>
</tr>
<tr>
<td>Srinivasan (1979)</td>
<td>India</td>
<td></td>
<td>100 to 2,100</td>
<td></td>
</tr>
<tr>
<td>Watanabe et al. (1977)</td>
<td>Philippines</td>
<td>177</td>
<td>24,000</td>
<td>Gloeotrichia bloom</td>
</tr>
</tbody>
</table>

aData extrapolated on the basis of 95% water content.
depending on the climatic conditions (Roger and Kulasooriya, 1979). Maximum algal biomass was noticed about 2 weeks (Kurasawa, 1956) and one month (Ichimura, 1954) of transplanting. From Rušia, Prikhod'kova (1968) observed maximum algal biomass just before tillering. In Senegal, Reynaud and Roger (1978) found maximum algal biomass between tillering and panicle initiation. In wetland fields of India, maximum biomass occurred a little later than in Senegal (Gupta, 1965). In Philippines, algal density was highest after heading during dry season, but during wet season, maximum biomass occurred after heading stage which was attributed to the availability of light (Watanabe and Lee, 1977; Watanabe et al., 1978b). Subsequent, decrease of biomass during the cultivation cycle was either due to the consumption by grazers (Kurasawa, 1956) or due to a deficient availability of light to the algae beneath the rice canopy (Kurasawa, 1956; Ibrahim et al., 1971).

6.3 Algal nitrogen contribution

Results of the field experiments depicted substantial nitrogen fixation by blue-green algae in the paddy fields. At different localities the nitrogen fixed in kg/ha/crop was 18-33 in Philippines (Alimagno, 1974),
30 (Watanabe and Lee, 1977) and 11 (Watanabe et al., 1978a), Japan, 26 (Hirano, 1958), Senegal 0-30 (Reynaud and Roger, 1978), Mali 50-80 (Trarore et al., 1978), India 14-53 (Singh, 1961) and 48 (Singh, 1976).

6.4 Epiphytic nitrogen fixation

Epiphytic blue-green algae also contributed to the fertility of deep water paddy fields. Occurrence of seven epiphytic nitrogen-fixing algae was reported from Mali (Martinez and Catleng, 1978). These were Anabaena tolurosa, A. vaginicolia, Cylindrosporum linciforme, Gloecapsa guatemita, Gloeotrichia natans, Hapalosiphon stuhlmanii and Nostoc sp. Kulasooriya et al. (1980a,b, 1981) reported that nitrogen-fixing population of Nostoc sp., Anabaena sp., Calothrix sp., Gloeotrichia sp. having a biomass of $3 \times 10^4$ colonies/g fresh paddy material contributed 10-20 kg nitrogen/ha/crop. Kulasooriya et al. (1980a) reported epiphytic occurrence of Gloeotrichia sp. on green algae.

6.5 Availability of fixed nitrogen to rice

Roger and Kulasooriya (1980) reviewed that nutrients fixed by BGA are released through microbial decomposition after the cells die or through exudation. Laboratory
studies indicated liberation of large portions of assimilated nitrogenous substances by BGA but under field condition no information is available. The principal means of nitrogen available to the rice crop is due to microbial decomposition after the death of the algae depending on the physiological stage of the algae, composition of associated microflora, suitability of the cell wall and relative biodegradabilities of specific components of algal walls. Bacterium like \textit{Bacillus subtilis} is found to decompose several $N_2$-fixing BGA rapidly by converting about 40 per cent of BGA N to ammonia in 10 days (Watanabe and Kiyohara, 1960). Singh \textit{et al.} (1981) and Saha \textit{et al.} (1982) observed that N release inform of ammonia was 12-35 per cent.

$^{15}$N enriched \textit{Gloeotrichia} sp. when incorporated into the soil, the recovery by the rice was 14.7 per cent in the first crop and 2.3 per cent in the second crop (IRRI, 1979). Wilson (1980) recorded that rice crop absorbed N from $^{15}$N labelled \textit{Aulosira} sp. and 51 per cent from the algae incorporated into the soil which shows that BGA nitrogen is readily available to rice.
II.7 Algalization

Rice is the major cereal crop whose nitrogen economy is maintained by the blue-green algae. De (1939) and Singh (1942) recognised the significant contribution of blue-green algae to the natural fertility in the tropical paddy fields. Use of blue-green algae was termed as "Algalization" (Venkataraman, 1961). Voluminous literature is available about the effectiveness of algae. Algalization in pot experiments is not so much reliable as it does not represent the true system (Dawson, 1967; Roger and Kulasooriya, 1980).

7.1.1 Algalization with inoculation

The beneficial effect of algal inoculation has been demonstrated at a number of places in terms of grain yield of many rice cultivars (Table 3).

7.1.2 Algalization by indigenous BGA

The need for encouraging indigenous blue-green algae has been stressed (Stewart et al., 1979). However, much prior to that work, this aspect has been done by amending phosphorus and other required nutrients to stimulate the native blue-green algae present in the
Table 3. Some reported response of rice plants to inoculation with BGA in field conditions (modified from Stewart et al., 1979)

<table>
<thead>
<tr>
<th>References</th>
<th>BGA</th>
<th>Treatment</th>
<th>Increase in grain yield(%)</th>
<th>Rice variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subrahmanyan et al. (1965c)</td>
<td>Mixture of Nostoc, Anabaena, Scytonema and Tolypothrix (200 g dry wt./ha)</td>
<td>a) Control (no additions)</td>
<td>-</td>
<td>Ptb 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) + algae</td>
<td>22.1</td>
<td>T 141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) + (NH₄)₂SO₄ (20 kg N/ha)</td>
<td>371.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) + lime (1000 kg/ha)+P₂O₅ (20 kg/ha)+Na molybdate (0.28 kg/ha)</td>
<td>67.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>e) + lime+P₂O₅+Na molybdate+algae</td>
<td>121.3</td>
<td></td>
</tr>
<tr>
<td>Aboul-Fadl et al. (1967)</td>
<td>T. tenuis (100-200 g dry weight/feddan)</td>
<td>a) Control (no additions)</td>
<td>-</td>
<td>Not given</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) + algae</td>
<td>16.6-19.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) + (NH₄)₂SO₄ (10-20 kg N/feddan)</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) + P₂O₅ (15 kg/feddan)</td>
<td>15.6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e) + P₂O₅+(NH₄)₂SO₄ (10 kg N/feddan)</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>f) + algae + P₂O₅</td>
<td>20.1</td>
<td>contd...</td>
</tr>
<tr>
<td>Authors</td>
<td>Treatment Description</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Sankaram et al. (1967)</td>
<td>Mixture of Nostoc, Anabaena and Scytonema (5 g dry weight/ha)</td>
<td>a) Control (no additions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) + algae</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) + lime (10,000 kg/ha) + P₂O₅</td>
<td>45.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20 kg/ha) + Na molybdate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.28 kg/ha)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) + lime + P₂O₅ + Na molybdate + algae</td>
<td>81.2</td>
<td></td>
</tr>
<tr>
<td>Venkataraman and Goyal (1969)</td>
<td>Mixture of A. fertilissima, T. tenue, C. muscicola and Nostoc sp. (2.5 kg dry wt./ha)</td>
<td>a) Control (no additions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) + algae</td>
<td>22.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) + (NH₄)₂SO₄ (112 kg N/ha)</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) + (NH₄)₂SO₄ + algae</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a-d) all treated 89.7 kg P₂O₅/ha and 50.5 kg K₂O/ha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas (1977)</td>
<td>Anabaena torulosa and/or Nostoc 4</td>
<td>a) Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) + urea (80 kg N/ha)</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) + A. torulosa</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) + Nostoc 4</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>e) + A. torulosa and Nostoc 4</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a-e) all treated with P and K</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
soil and these developed algae have been used for better crop response. P was found to be limiting factor for the growth of these algae and confirmed in laboratory study (De and Mandal, 1956; Konishi and Seino, 1961; Relwani, 1963; Venkataraman, 1968; Wilson and Alexander, 1979). However, reports of field experiments are scanty. Nevertheless, the contribution of El-Nawawy et al. (1958), Relwani (1963), Jha et al. (1965), Mahapatra et al. (1971), El-Nawawy and Hamdi (1975) and Cholitkul et al. (1980) are noteworthy. Subrahmanyan (1972) reported good growth of several species of indigenous blue-green algae such as Nostoc sp., Tolypothrix sp., Anabaena sp. He further observed that indigenous algal blooms proliferated by the amendment of lime, superphosphate, molybdenum which increased the grain yield and nitrogen uptake upto 11.5 kg/ha under field conditions.

7.2 Algalization with nitrogen fertilizers

Much controversy exists about the nature of algalization with nitrogen fertilizers. The failure of algalization has been reported and explanation offered was inhibitory activity of applied inorganic nitrogen to BGA (Watanabe, 1973; Sankaran, 1975, 1977).
However, Aiyer (1965) explained that poor grain yield due to algalization (*Trichothrix tenuis*) with ammonium sulphate. De and Sulaiman (1950b) observed the growth of rice and alga *Trichothrix* sp. when N fertilizer was applied after 3-4 weeks of transplantation.

On the other hand, several reports indicate the beneficial effect of algalization in the presence of fertilizer nitrogen. Aiyer *et al.* (1972) observed uniform beneficial effect of algalization with increasing levels of urea fertilizer N. Similar results have also been obtained even at N levels as high as 120 kg N/ha (Goyal and Venkataraman, 1970; Venkataraman, 1979). Effective algalization with increasing doses of N fertilizer has been attributed due to the production of growth promoting substances (Venkataraman, 1979). Roger *et al.* (1980) found that the surface application of N decreased the nitrogen-fixing ability of the algae, however, by placement of N fertilizer deep in soil, did not decrease the algal N fixing capacity.

7.1.4 Effective algalization through cultural practices

The influence of agronomic practices on the growth of BGA has been reported. Shioiri and Mitsui (1935) .
demonstrated the rate of decomposition of algae in the soil. Singh et al. (1981) reported BGA suitable for use as a green manure as compared to higher plants. Tillage increased ammonification and mid season tillage increased phosphorus, iron availability which favour the growth of BGA (Roger and Reynaud, 1979). Superficial incorporation of algae in the soil, led to the recolonization of the submerged water (Roger and Reynaud, 1979). Saha et al. (1982) revealed that incorporation of Aulosira sp. could appreciably change the nitrogen and phosphorus availability which might influence the growth and nutrition of rice.

7.3 Effect of algalization on grain yield

Effect of algalization on grain yield under field conditions has been reported in China (Ley, 1959; Academia Sinica, 1978), Egypt (El-Nawawy et al., 1958; El-Nawawy and Hamdi, 1975), Japan (Hirang et al., 1955; Watanabe, 1961, 1962, 1965), Philippines (Pantastico and Ganzales, 1973) and in India (Singh, 1961; Jha et al., 1965; Venkataraman and Goyal, 1969; Lehri and Mehrotra, 1970; Venkataraman, 1972, 1975, 1979) (Table 3). Several trials conducted at CRRI, Cuttack yielded encouraging results with regard to algal contribution.
to paddy under field conditions (Relwani, 1963, 1964, 1965; Relwani and Subrahmanyan, 1963; Sankaram, 1971; Sankaram et al., 1966, 1967; Subrahmanyan, 1972; Subrahmanyan et al., 1964a,b, 1965a,b,c; Subrahmanyan and Manna, 1966; Singh, 1974a, 1979, 1987).

7.4 Algalization on yield components

Algalization has also been known to increase number of effective tillers, straw yield and other yield components (Watanabe, 1962; Singh, 1961; Subrahmanyan and Manna, 1966; Aiyer et al., 1972; Alimago and Yoshida, 1975; HIH, 1977).

7.5 Nitrogen uptake by rice

Based on the nitrogen uptake by paddy in field condition, with and without algal inoculation (Sundara Rao et al., 1963; Hosada and Tanaka, 1955), studies conducted earlier at CRRI, Cuttack established in field trials that BGA contributed yield equivalent to 20 kg N/ha (Sankaram, 1967). Subrahmanyan (1972) explained higher N-uptake by paddy due to algalization at each crop showing gradual rise in successive algalized crops. Similar observations were made in Japan by Yamaguchi (1979) from 1952 to 1956 who concluded increase in
N-uptake by paddy in algalized plots. However, Alimagno and Yoshida (1975) from Philippines reported a low value of N-uptake.

7.6 Residual effect

The successive algalization trials have been found to enrich soil fertility as evident from published work. Watanabe (1962) reported the relative increase of grain yield by 2, 8, 15, 19.5, 10.6 per cent in successive years from 1956 for 5 years. In Japan, it is reported to increase the grain yield ranging from 5 to 20 per cent in four successive years (Hirang et al., 1955). Watanabe (1956) observed 2.7-21.8 per cent yield increase in four successive years. Watanabe (1962) further noted that the increase ranged from 2 to 19.5 per cent till fourth year and then in the fifth years it declined to 10.6 per cent. Studies conducted at CRRI, Cuttack indicated increase in grain yield to the tune of 8-43 and 21-41 per cent in control and treated plots during second year (Relwani, 1964). These results were further confirmed by Sankaram (1971) and Subrahmanyan (1972).
II. 8 Effect on soil properties

8.1 Soil N

BGA increased the nitrogen content of the soil (De and Mandal, 1950a; Watanabe, 1962; Aiyer, 1965; MacRae and Castro, 1967; Chopra and Dube, 1971; Subramanyan, 1972; Alimago and Yoshida, 1975; Singh et al., 1981; Saha et al., 1982). However, the algal inoculation in successive crops had no appreciable effect on the N content of the soil as reported by Sankaram (1971) and Aiyer et al. (1972). Algalization resulted in organic nitrogen availability beyond the tillering stage of the crop and no depression in addition of organic N due to algae was observed even in presence of chemical nitrogen (Chopra and Dube, 1971). BGA also increased the available nitrogen as expressed by the amount of ammonia produced (Sankaram, 1967; Singh et al., 1981; Saha et al., 1982).

8.2 Organic matter

In long term experiments of cumulative and residual effects of algalization, a gradual increase in organic carbon has poorly documented. Singh (1961) reported an increase of 68.7%
organic matter in 'Usar' soils. Arora (1969) reported increase of organic matter at successive algal inoculation. The work conducted at CRRI, Cuttack, India revealed that the organic matter content of the soil gradually increased (Subrahmanyan et al., 1965c; Sankaram, 1971; Saha et al., 1982). On the contrast, no appreciable increase in organic matter content in the rice field soils has been reported (Aiyer et al., 1972) of Kerala.