XI. DISCUSSION
DISCUSSION

Taxonomic account:

The present work was undertaken with a view to screen the blue-green algal flora of different crop fields in Maharashtra. Blue green algae are known to play an important role in nitrogen economy (Rodgers et al., 1979; Venkataraman, 1981). Therefore, in order to improve soil fertility, extensive search to select, isolate and develop blue green algal strains having more capacity to fix atmospheric nitrogen is needed (Watanabe, 1965). Air dried soil samples were collected from different fields up to the depth of 6 inch and nitrogen fixing blue green algal flora was studied by serial dilution technique using Fogg's nitrogen free semi-solid medium. Serial dilution technique to enumerate soil algal flora have also been employed by various workers (Gonzalves and Yalavigi, 1959; Allen, 1973). In the present investigation a total of 30 samples were analysed and all the samples showed the presence of blue green algal forms. Quantitatively there was quite a large
variation in the algal number per gram of soil. Samples from cotton, wheat and sugarcane showed maximum number (more than 1000/gm oven dry soil) of algae when compared with others. The variation in the number was also seen between two samples of the same crop from different places. Esnarch (1914) and other workers (Allen, 1973; Bharati and Bongale, 1975) also recorded such types of variations in cultivated soils and attributed them to the crop manuring, availability of water and physico-chemical properties of soils at various places. Soil samples used in the present study showed such variations as follows (percent):

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<tr>
<td>Clay</td>
<td>2.5</td>
<td>3.9</td>
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<tr>
<td>Silt</td>
<td>1.5</td>
<td>4.0</td>
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<tr>
<td>Fine sand</td>
<td>41.8</td>
<td>57.0</td>
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<tr>
<td>Coarse sand</td>
<td>36.2</td>
<td>56.7</td>
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<tr>
<td>pH</td>
<td>7.7</td>
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<tr>
<td>Organic matter</td>
<td>5.0</td>
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<tr>
<td>N</td>
<td>0.03</td>
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<tr>
<td>P</td>
<td>0.6</td>
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K ... 0.08 - 0.11
Na ... 0.24 - 0.31
Ca ... 0.22 - 0.58

Qualitatively a total of 41 blue green algal species belonging to 19 genera were recorded during this study. Most of them confined to Nostocales (27 species belonging to 14 genera) while few to chroococcales (3 spp.) and Stigonematales (5 spp.). An examination of the list of algal species isolated from each soil sample range from a minimum of 2 spp. to a maximum 28 spp. Not only that but percentage of each species also varied from sample to sample. The results are in agreement with various workers who have reported different number of species from different field soils in India and abroad. (Kaisi, 1976; Rushforth et al., 1976; Ali et al., 1973; Rao, 1936; Dixit, 1936; Vasistha, 1963; Gupta, 1965; Lata, 1965; Marathe, 1967; Vaidya and Patel, 1968; Tiwari, 1972; Singh, 1973; Bharati and Bongale, 1975; Prasad et al., 1977; Bongale, 1981). All of them advocated that the physico-chemical properties of each soil sample might be responsible for this variation. Variation
in the number of species in the samples collected from different fields in the present investigation might be due to the physico-chemical properties of the soil through their exudation. Thus, Gonzalves and Yalavigi (1959) showed qualitative and quantitative variation in the algal forms in the rhizosphere soil of jowar, wheat and cotton. Katzenelson (1946) also found that blue greens are more abundant in the rhizosphere of mango. Moreover, the methods of study and type of media used also reflect on the number of algal species from different samples. Occurrence of more algal colonies per gram of soil from sugarcane, wheat and cotton might be attributed to heavy irrigation when compared to other rainfed crops.

Qualitatively species of *Nostoc* and *Anabaena* were more abundant when compared with other genera; *Nostoc hatei* was recorded in maximum number of samples. Similarly, *Anabaena spiroides* was found to be present in 7 samples of soils collected. *Anabaena fertilissima* was recorded in 6 samples while, *Tolypothrix tenuis, Westiellopsis prolifica, Fischelerla muscicola, Oscillatoria animalis,*
Anabaena ballyvangalii and Aulosira prolifica were found to be present in 5 soil samples. This was followed by Oscillatoria foreaui, Oscillatoria animalis, Phormidium orientale, Anabaena variabilis, Anabaena sp., Nodularia sp. and Scytonema myochrous which were found to be present in 4 soil samples collected from different places of this region. Nostoc calcicola, Cylindrospermum sp., Microchaete sp., Haplasiphon welwitschi, Calothrix marchica, Calothrix sp. were found to be rare in their occurrence.

It may be stated that certain factors needed for the appearance of rare species were not available under cultural conditions or that the environment in which the alga was growing under natural conditions was not present under cultural conditions. Among the algal species recorded in the present study all have been reported by various workers from different soils all over the world (Table 2) except few like Microcystis elabens, Nostoc hatei, Anabaena ballyvangalii and Tolypothrix fragalis. These four species have been, however, reported from
other aquatic habitat other than the soil and hence may be considered as new records from the Indian soils. *N. elabens* have been reported from sand filters (Ganapathi, 1940a), *N. hatei* from stagnant ponds (Dixit, 1936), *A. ballyganglii* from plankton (Banerji, 1938) and *T. fragalisa* on white washed walls (Rao, 1937). In addition, some species of *Cylindrospermum, Nostoc, Fortica, Calothrix* show slight morphological variations and are new records from Marathwada soils.

**Effect of agrochemicals on nitrogen fixing blue green algae Nostoc hatei:**

Biological nitrogen fixation is a significant factor in global nitrogen cycle. The largest part of biological nitrogen fixation, more than 25% is attributed to soil microorganisms and blue green algae have also been recognised as an important component in nitrogen fixation (Arnon, 1955; Singh, 1961; Fogg et al., 1973; Bharati and Bongale, 1975; Burns and Hardy, 1975; Stewart, 1975; Venkataraman, 1981). *Nostoc hatei* was found to be more prevalent in these soils.
prevalent in these soils and hence was selected to investigate whether its nitrogen fixing capacity and growth was affected by different agrochemicals used in crop management. Various workers employed different methods for growth and nitrogen fixation. Some of them used dry weight of algal mass (Padhy and Pattnaik, 1974; Gangawane, 1978; Das and Singh, 1979; Rohwer and Fluckiger, 1979; Bharati and Angadi, 1981) while the others used optical density of cells or acetone soluble pigments (Torres and O'Flaherty, 1976; Rai and Singh, 1977; Hawxby et al., 1977). Sorokin (1973) very well discussed the utility of these two methods. Dry weight of algal biomass and O.D. of acetone soluble pigments were used as a criterion for growth measurement of this alga grown in the N-free Fogg's medium incorporated with various compounds. Acetylene reduction technique, supposed to be most suitable (Tu, 1978, Rohwer and Fluckiger, 1979) could not be employed during the present investigation due to lack of facilities. However, nitrogen content in the algal biomass as well as in the culture fluid was estimated by Microkjeldahl technique as also has been used by many workers
(Singh, V.P. 1979; Kapoor and Sharma, 1979; Grover and Puri, 1979 and Tiwari et al., 1981).

Antibiotics: Of the four antibiotics, Aureofungin was inhibitory at 100 ppm for growth, nitrogen fixation and heterocyst formation while Agrimycin, Plantomycin and Streptocycline were highly toxic to this alga. The results are in agreement with Gangawane (1973) who showed that Nostoc sp. could grow up to 100 ppm of Aureofungin while Streptocycline was toxic even at 1 ppm. He also found that other blue greens like Westieliopsis sp. Aulosira, Tolypothrix sp. and Calothrix sp. grew up to 100 ppm of Streptocycline. Agrimycin and Plantomycin used in this investigation also contain Streptomycin along with Chlortetracycline and hence might have inhibited Nostoc hatei completely even at lowest concentration. This may be attributed to the fact that these antibiotics are selectively toxic to prokaryotes and inhibits the growth by causing misleading of genetic message during protein synthesis. On the contrary, Aureofungin an antifungal antibiotic is selectively toxic to eukaryotes (Stainer et al., 1971).
Fungicides: Perusal of the results indicate that all the fungicides (Alitte, Agrosan, Bavistin, Brassicol, Captan, Dithane-Z-78, Dithane-M-45, Emisan, Fytolan and Thiram) used in this study inhibited the growth, heterocyst formation and consequently nitrogen fixation. Inhibition concentration, however, varied according to the fungicide. For example, Alitte and Bavistin inhibited alga at 100 ppm while others like captan, Dithane Z-73, Dithane M-45, Fytolan at 1-50 ppm only. Agrosan, Emisan and Thiram did not support the growth at all indicating more toxicity.

Inhibition of blue green algae by different fungicides have been reported by different workers (Fitzgerald et al., 1952; Whitton and MacArthur, 1967; Zweig et al., 1968; Anand and Veerappan, 1974; Gangawane and Saler, 1979; Gangawane, 1980; Bharati and Angadi, 1980). Genetical and biochemical background of algal growth inhibition due to fungicide has not worked out since so far. However, Wegler (1970) suggests that inhibition of growth, nitrogen fixation and oxygen production seem to proceed simultaneously and initial effect is on the process of photosynthesis. In the
present investigation inhibition of growth/nitrogen fixation and heterocyst formation might be attributed to the direct effect of fungicidal compounds at higher concentrations which act as photosynthetic inhibitors.

It is interesting to note that some of these compounds proved to be stimulatory for growth, nitrogen fixation and heterocyst formation. Thus, Aliette stimulated growth of alga at 1-50 ppm. Captan stimulated growth, nitrogen fixation and heterocyst formation at 1 ppm while Dithane-M-45 and Fytolan stimulated growth and nitrogen fixation respectively. Stimulation of growth might be attributed to the active metabolism and adaptation of this alga in the presence of these fungicides. Microbial degradation of fungicides is also well known (Alexander, 1969). Stimulatory effect of fungicides on *Anabaena ovales*, *Aulacoseira fertilissima*, *Nostoc* sp. and *Tolypothrix tenuis* have been reported by some workers (Venkataraman, 1971; Gangawane and Saler, 1979; Gangawane, 1980; Bharati and Angadi, 1980).
Fertilizers: Exposure of *Nostoc hatei* to fertilizers showed inhibition of growth over 500 ppm of N, P alone or NPK in combination. Lower concentrations, however, (10-50 ppm) were found to be stimulatory for growth. Nitrogen fixation was inhibited by Urea and NPK at lower and higher concentrations; one ppm of Superphosphate proved stimulatory. Same was the effect on heterocyst formation due to the use of fertilizers. Studies on these lines have been carried out by Stewart (1969) and Singh (1961, 1975). Stewart et al., (1968) showed that higher concentration of nitrogen in the medium reduce the heterocyst formation and consequent to which nitrogenase activity was reduced in certain blue green. Singh (1975) observed that higher concentration of Urea and Ammonium sulphate (0.1 mg/ml) showed reduction of growth and heterocyst formation in about ten blue green algal species including *Nostoc carneum* and *Nostoc* sp. Suppression of heterocyst formation and nitrogenase synthesis at the higher concentration of nitrogenous compounds might be the possible reason for the reduction of growth and nitrogen fixation by *Nostoc hatei* in the present study. There are several
evidences that heterocysts are the site of nitrogen fixation (Fogg, 1949; Stewart, 1975). Higher concentrations of superphosphate is tolerated by heterocyst.

Herbicides:

All the three (Basalin, Tolan and 2,4-D) herbicides proved to be inhibitory for growth, heterocyst formation and consequently to nitrogen fixation. Tolan was more inhibitory when compared with Basalin and 2,4-D. There are several reports on the effect of herbicides on growth and nitrogen fixation by different blue green algae (Gamble et al., 1962; Hamdi et al., 1970; Inger, 1970; Venkataraman and Rajyalakshmi, 1971; Singh, 1974; Wright et al., 1977; Khalil et al., 1980). All of them reported that different species of blue green algae including Nostoc spp. are sensitive to herbicidal compounds including 2,4-D Basalin and Tolan. However, Inger (1970) and Ahmed and Venkataraman (1971) reports that there is no effect on Nostoc muscorum and Nostoc punctiformae of MCPA and MCPB at the doses recommended for field application. Compounds
used in the present study may also not harmful to *Nostoc hatei* in the field conditions at these levels. Potential physiological and genetic hazards caused by these compounds might be responsible for the reduction of growth and nitrogen fixation in this alga as has been suggested by Amla and Kochhar (1982). In addition, Grossbard (1976) Hawxby et al. (1977) attributes it to inhibition of photosynthesis in these organisms.

**Plant growth hormones:**

It seems that plant growth hormones are stimulatory at the lower concentrations while inhibitory to higher concentrations for the growth, nitrogen fixation and heterocyst formation. Thus, in the present study Selmone, Planofix, IAA and Kinetin stimulated the growth of *Nostoc hatei* from 0.5 to 10 ppm. Gibberlic acid, however, showed its stimulatory effect from 0.1 to 100 ppm on the growth. Again, Selmone, IAA and GA₃ were found to be favourable for nitrogen fixation but not the Kinetin or Planofix. Heterocyst formation was reduced considerably due to all
the compounds except GA$_3$ at 0.5 to 1 ppm. The stimulatory effects of hormones at lower concentrations might be attributed to their (hormones) influence on the various intracellular enzymatic levels and subsequently to the interference with selective utilization of food material from the medium. Bongale (1973) working with 
_Haplosiphon welwitschii_ also showed that IAA and GA promote growth at lower concentrations. Similar results have been reported by various workers (Ahmed and Winter, 1968; Padhy and Pattanaik, 1976; Adhikary and Pattanaik, 1978) in case of different blue green algae and hormones.

**Micronutrients:**

Mineral requirements of blue green algae are not different from the requirements of other photosynthetic plants. In the present investigation effect of Molybdenum, Copper, Manganese, Iron, Zinc and Boron on growth and nitrogen fixation in _Nostoc halei_ was studied. Growth of this organism was stimulated by all the micronutrients but at certain level of concentrations. Thus Molybdenum, Zinc,
Boron and Iron were stimulatory upto 10 ppm, while Copper and Manganese at or more than 50 ppm. These elements proved to be stimulatory for nitrogen fixation as both the extracellular and intracellular nitrogen was increased. Inhibition of heterocyst formation was seen at more than 50 ppm. Micronutrient requirements of algae have been very well reviewed by Eyster (1966) and concluded that Manganese, Iron, Boron and Molybdenum are required for nitrogen fixation in blue green algae. However, critical concentration of these elements only support these activities. This might also be the reason in the present study why at certain concentration of micronutrients _N. hategi_ has been stimulated. Iron and Molybdenum are the components of nitrogenase enzyme complex and Boron accumulates certain soluble nitrogenous compounds. Copper has been shown to be essential for nitrogen fixation in _Rhizobium_ and _Azotobacter_ by Hall Sworth et al. (1960) while in _Anabaena deliolum_ by Rahul and Singh (1980). Induction of nitrogenase and nitrate reductase, enzyme might have produced some metabolites which detoxified copper in the present organism employed in this study. Stimulation or inhibition
of growth and nitrogen fixation in the presence of different micronutrients have been reported by some workers (Koelling, 1971; Gleason and Wood, 1976; Kapoor and Sharma, 1979; Rahul and Singh, 1980).

**Insecticides, Rodenticide and Nematicide:**

Altogether 17 insecticides, one rodenticide and one nematicide were tested in the present study. Rodenticide Zinc phosphide was inhibitory for the growth, nitrogen fixation as well as heterocyst formation while the nematicide Dasanit showed its inhibitory effect at 500 ppm but 1 and 10 ppm were found to be stimulatory for nitrogen fixation in *Nostoc halui*. Among the insecticides majority showed their inhibitory effect at varying concentrations. Aldrin, BPMC, Lindane, Pyrethrum and Thimet were stimulatory for growth at lower concentrations (1-10 ppm); BHC was stimulatory for both, the growth and nitrogen fixation at 1-100 ppm. Cyathion also promoted growth, nitrogen fixation and heterocyst formation at 1-10 ppm. Rogor was only stimulatory to nitrogen fixation (50 ppm) while Thimet to heterocyst formation (1-10 ppm). The results in the present
study agree with the earlier workers (Raghu and MacRae, 1967; Vance and Drummond, 1969; Singh, 1973; Ahmed and Venkataraman, 1973; Rohwer and Fluckiger, 1979; Tarar and Salpekar, 1979, Kar and Singh, 1979; Das and Singh, 1979; Gangawane and Deshpande, 1981; Subramanian, 1982) who showed both stimulatory or inhibitory effects of different insecticides on different blue green algae including some Nostoc sp. at various concentrations. Thimet was found to be toxic even at 1 ppm to Nostoc sp. (Gangawane, 1979), while this species also did not grow beyond 10 ppm of Sevidol and Zolone (Gangawane et al., 1981). It is interesting to note in the present investigation that there was quite a large variation in the insecticidal compounds and their concentrations those could stimulate or inhibit the growth, nitrogen fixation or heterocyst formation in Nostoc hatei suggest that specific insecticide affect specific metabolic process of this alga.

Algalization and rhizosphere microflora of rice:

Algalization with Nostoc hatei did not show any significant variation in the rhizosphere mycopopulation.
However, at the initial stage of growth period there was slight increase in the population both in the rhizosphere and soil. Bacterial population was increased due to algalization. Of the 21 fungal species, different species responded differently to the algalization. For example, *Absidia corymbifera*, *Penicillium* sp., *Aureobasidium pullulans* etc. were stimulated while *Curvularia lunata*, *Aspergillus terreus*, *Neocosmospora vasinfecta* etc. were inhibited in rhizosphere or soil. There are reports that bacterization with *Rhizobium* or *Axotobacter* influence the mycofloral population both quantitatively and qualitatively in the rhizosphere of various plants (Holland and Parker, 1966; Dey et al., 1968; Patel, 1969; Gangawane and Deshpande, 1974). They advocate that increased exudation of substances from the roots of inoculated plants influence the rhizosphere mycoflora in different ways. Thus, in the present investigation increase, decrease or even neutral effect on some fungi in the rhizosphere of rice due to algalization might be at the consequence of exudates not only from the plants but also algal secretions. Lakshmi-kumari et al. (1972) have shown that *Asotobacter* spp.
produced a thermolabile ether soluble substance fungistatic to *Fusarium moniliforme*. Whether such types of compounds are secreted by the alga *Nostoc hatsch* in the rhizosphere and their study of possible effects will throw much light in this connection. Studies on the effect of algalization on the rhizosphere mycoflora of rice showed that all the four pesticides (Streptocycline, Phorate, Bavistin and Melathion) exerted their influence differently. Streptocycline and Melathion did not affect the total fungal population as they are not fungicides. Phorate and Bavistin exerted reduction in the total population in the rhizosphere. However, when individual species are taken into account they responded differently to each individual pesticidal compounds in the rhizosphere or in the soil. For example, *Absidia corymbifera, Aspergillus niger, A. nidulans, A. terreus, A. ustus, Penicillium funiculosum* showed their increased percentage frequency over the control indicating their stimulation in the rhizosphere or soil due to the application of these compounds. On the contrary, some of them were reduced while others were unaffected. Selective
action of pesticides to the microflora is well known for not only of the compounds with specific spectrum but also with broad spectrum effectiveness of the compounds. (Byrde and Richmond, 1976; Bollen, 1979). Moreover, the physiological changes in the roots might have reduced the inhibitory effect of these pesticidal compounds and stimulation might have occurred (Kutznelson and Richardson, 1943). Presence of organic nutrients in the soil is also known to enable microorganisms to overcome fungistatic effects in soil (Martin, 1950). Reduction in the fungal population in the rhizosphere or soil of various crop plants due to pesticidal treatments is also reported by several workers (Alexander, 1969; Suillia, 1969; Gangawane and Deshpande, 1979; Bollen, 1979).

**Effect of Nostoc hatei on soil microflora:**

In order to see the effect of alga on soil microflora in the presence of pesticidal compounds artificial rhizosphere technique was adopted. This gave the influence of alga on soil microflora in various ways. Rhizosphere micropopulation
was always higher when compared with control soil. Algal exudates from the cellophane bag create an artificial rhizosphere which may have attributed the higher population analogous to the higher plant roots. Influence of root exudates on soil microflora is well known (Rovira, 1956; 1965; Gangawane and Deshpande, 1973). Similarly, exudation of different organic substances by blue green algae in soil is reported (Venkataraman, 1964; Singh and Trehan, 1973; deCano et al., 1979; Puri and Grover, 1981).

Application of different pesticidal compounds influenced microfloras in various ways. Streptocycline, 2,4-D and Bavistin increased the bacterial and fungal population in the artificial rhizosphere while Melathion reduced; Phorate had more or less no effect. Organic compounds from algae might have nullified the toxic effect of these compounds. Qualitatively, however, individual species responded differently. Most interestingly Fusarium moniliformae, the potential pathogen of rice was inhibited in the rhizosphere or soil due to all the compounds whereas other saprophytes (beneficial) were
increased or unaffected. The probable explanation for this is that different pesticidal compounds has different fungicidal spectra as has been suggested by Saler (1981).

Aerocchemicals and Production of Ascorbic acid:

The requirement of L-ascorbic acid in various metabolic pathways and its biosynthesis in algae and higher plants is well known (Liso et al., 1978). Results in the present study indicate that some compounds are stimulatory while some of them are inhibitory.

For the production of ascorbic acid by Nostoc haitai, for example, Urea, Gibberillic acid, Indole Acetic acid, Kinetin, Boron, Zinc, Copper, Molybdenum, Iron, Aldrin, BHC, Cyathion, Carbofuran, Thimet, Rogor, Bavistin, Fytolan, Captan, Desanit etc. were stimulatory in the production of ascorbic acid while Superphosphate, Agrimycin, Plantomycin, Streptocycline, BPMC, Sevin, Dithane-Z-78, 2,4-D, Zinc phosphide etc. were inhibitory. The stimulation or inhibition, however, was related to the concentration of compounds used. For instance, concentration of micronutrients
more than 1 ppm reduced both the growth and ascorbic acid production in this alga. Ascorbic acid function as one of the biological oxidation reduction substances. It is oxidized to dehydro-L-ascorbic acid by the enzyme ascorbic acid-oxidase or by certain other oxidative enzymes. It is, therefore, probable that their might be direct or indirect influence of these compounds on oxidative enzyme system of N. hatei as has been suggested by Gangawane and Datar (1978) in case of tomato germplasm. Moreover, major contribution of blue green alga towards the growth of rice plant is through the production of extracellular productions together with nitrogen fixation. Stimulation of ascorbic acid production in this alga by some agrochemicals may have certain beneficial effect on the growth of rice plant in the field conditions.

Nostoc hatei and production of enzymes by soil fungi:

Extracellular production of cellulolytic and pectolytic enzymes by soil fungi is an important tool not only in the degradation of organic matter but also in the pathogenesis.
Culture filtrates from *N. hateli* influenced these enzymatic activities in different fungi in different ways. There was stimulation or inhibition of the production of these enzymes. For instance, PG, PMG and CX activity were stimulated in *Helminthosporium tetramera* while reduced in *Trichoderma viride* and *Rhizopus stolonifer*. In *Aspergillus niger*, PG was stimulated while PMG and CX were inhibited. Organic compounds in the algal filtrate might be responsible for this. Detailed biochemical analysis of the algal filtrate will throw more light as to why certain enzymes are stimulated while others suppressed in particular fungus. However, the study indicate that in the presence of alga there may be direct effect on the degradation of organic matter in the rice field which is always favourable for the growth of alga.

Linear growth of *H. tetramera*, *Aspergillus flavus* and *A. niger* was stimulated while there was inhibitory effect on *Rhizopus stolonifer* and *Trichoderma viride*. 
Overall perusal of the the results in the present investigation indicate that soils at different places of Marathwada region harbour rich blue-green population; some of them being effective nitrogen fixers like *Nostoc* *hatei*. Use of agrochemicals seems not to be harmful in the field level although *in vitro* certain compounds were found to be harmful for growth and nitrogen fixation. Interestingly at lower concentrations some of them show beneficial effects. Maximum concentration of compounds used in the present study was 500 ppm, which in certain cases was inhibitory to the alga. However, this much of concentration may not reach to the soil and even if it reaches decomposition with different soil factors (Soil type and soil structure, pH, temperature, moisture, microbial population etc.) lowers concentration to the minimum. According to Chopra (1971) 45-80% pesticides disappears from the soil within a year. Hence use of agrochemicals alongwith algal biofertilizers can be recommended. Algalization exerts considerable influence on the rhizosphere and soil microflora alone and in combination with pesticides. Moreover, there is interaction between algae and other microorganisms in the soil.
Thus, the rhizosphere of rice can also be manipulated with different pesticide treatment in order to get better growth and yield.