PADDY FIELD CYANOBACTERIA: DIVERSITY AND PESTICIDE TOLERANCE

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By

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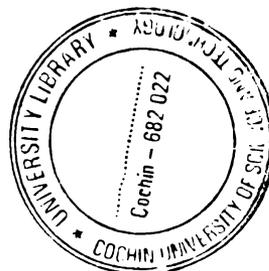
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November, 2004

DECLARATION

I, R.Ramesh, hereby declare that the results presented in this Ph.D. thesis entitled 'Paddy field cyanobacteria: Diversity and pesticide tolerance' are based on original research work carried out by me under the guidance of Dr.Ammini Joseph, Professor, School of Environmental Studies, Cochin University of Science and Technology and that no part of this work has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title or recognition.

Kochi
November, 2004.

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Certificate

This is to certify that the results presented in this thesis entitled 'Paddy Field Cyanobacteria: Diversity and Pesticide Tolerance' are based on the original research carried out by Mr.R.Ramesh, under my guidance in the School of Environmental Studies, Cochin University of Science and Technology in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy and no part of this work has previously formed the basis for the award of any degree, diploma, associateship, fellowship, or any other similar title or recognition.

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PREFACE

Microalgae comprise both prokaryotic and eukaryotic forms, occupying aquatic habitats, with quite a few abounding sub-aerial and aerial niche. The cyanobacteria which are prokaryotic algae have become accepted as a major group among Monera. The metabolic strategies of these organisms in exploiting aerobic and anaerobic photosynthesis, and the capability of surviving prolonged periods of drought results in its ability to thrive in widely fluctuating environments. Paddy fields of the tropics, because of the flooded conditions, and shade provided by the crop canopy, become a congenial ecosystem favouring the growth of cyanobacterial flora.

Although algae including cyanobacteria are autotrophic, plenty of organic matter in the soil can increase the algal incidence through increasing the availability of carbon dioxide as a consequence of decomposition.

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Presence of humic substances enhance algal population in soil, without acting as direct source of nutrients (Lee and Bartlett, 1976). Another closely related observation was that organic matter content increase algal incidence through increasing the moisture content of the soil (Friedman and Galum, 1974). Other factors like vegetational cover and cultivation practices affect distribution of algae in soil. While the physical and chemical status of the soil has a bearing on the fertility level of the soil, the productivity of the soil largely depends upon the availability of the nutrients, which in turn is regulated by the microbial population. Algae add substantial quantities of organic matter through primary production, and this increase the humus content of the soil. Most of the soil algae possess mucilaginous sheath which help in binding the soil particles together, increasing the size of the soil aggregates. This optimize aeration, water movement, root development and fertilizer utilization (Roychoudhary et al., 1979; Subhashini and Kaushik, 1981). Soil algae can also bind with Na^+ and K^+ ions and thus reduce the soil salinity (Subhashini and Kaushik, 1981). Algae are also reported to bring down the level of oxidizable matter in soil especially sulphate and iron content (Aiyer et al., 1971). Many algae have been found to be capable of solubilizing insoluble phosphate in the soil (Bose et al., 1971).

The microalgal cover crop is hardly visible and it needs good perseverance to realize the effect of this important self regenerating system. In spite of all, the existence of algae in paddy fields is not all that easy these days; for, sustainable paddy cultivation also means a sustainable pest management, and sustainable pest control depends heavily upon the use of

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pesticide products. Microalgae easily become the first, in the list of non-target organisms that are affected by pesticides.

Crop loss due to biotic stresses is a major menace to agriculture. During the past 50 years this has been controlled by the use of pesticides, though, it may not be counted as a good measure of sustainable practice. Pesticide use in India for crop protection and public health goes to the extent of 85,000 tons per year. Of these 77.8% are insecticides, and the rest fungicides, herbicides, rodenticides, fumigants and miscellaneous pesticides. Of this, paddy, which occupies about 24% of the crop area, accounts for 17.2% of the pesticides used (Maruthanayagam and Sharmila, 2004). The use of chemical crop protectants has transcended effects on environment even outside agro-ecosystem, on biodiversity, drinking water, and on life especially at the end of food chain including humans (Zadoks and Waibel, 2000).

Recognizing the importance of cyanobacteria as a fertilizer input and soil conditioner, it is attempted to document the flora of a unique ecosystem - the saline pokkali fields, and that of an upland paddy field, subject to agricultural activity. Environmental characters of pokkali fields apart from being suitable for paddy and prawns are ideally suited for proliferating growth of many soil microorganisms. Cyanobacteria constitute a prominent group among the soil organisms in the pokkali paddy fields, but the fields are so placed that they are low lying, adjacent to the sea, and prone to surface drain. Thus, even though the pokkali fields are pristine environments and cradle for cyanobacteria, the lullabies are chemical toxicity and salinity stress.

In this study, the population size of the microalge of two different types of paddy fields was investigated throughout two consecutive cropping seasons. Further the diversity of cyanobacterial flora was explored through enrichment culture procedure. The ecotoxicology of an isolated species of cyanobacterium and a green microalga were studied with respect to effect of pesticides. The effect of pesticide and salinity are studied in detail to reveal their impact on cyanobacteria of paddy fields.

The research is presented here in **5** chapters. In chapter **1**, enumeration of the soil microalgae in a low land paddy field (pokkali) and an upland paddy field are recorded. An account on cyanobacterial diversity also is presented along with.

Chapter **2** gives the characterization of the unicellular cyanobacterium *Synechococcus elongatus*, isolated from pokkali field. The organism was subjected to EM study, absorption spectra analysis, antibiosis test, GC analysis of nitrogenase and growth kinetics study. This chapter also deals with a detailed investigation on pesticide tolerance of the isolate. Four insecticides, three fungicides two herbicides and one bactericide, which are used commonly in paddy cultivation, were experimented. The experiments with cyanophycean member *S. elongatus* were repeated with a chlorophycean member *Chlorella pyrenoidosa*, and the results were compared.

Chapter **3** embodies three microcosm studies exploring three different eco-physiological properties of the isolate *S. elongatus*. In microcosm one, the changes brought about to the property of paddy field soil by algalization is described. The second microcosm experiment deals with abatement of

pesticide residue in soil upon algalization with the test organism. The measurement of pesticide residue was done with GC. The third soil microcosm experiment is about leachate toxicity. Leachates prepared from paddy field soil fortified with pesticides, were bioassayed for toxicity. The changes in leachate toxicity upon algalization of the paddy field soil with test species was the point of focus.

Chapter **4** discusses the interaction of pesticides with salinity. Different concentrations of pesticides were combined with different levels of salinity to produce 12 combinations. *S.elongatus* was cultured to find out the growth response under the combined stress conditions.

In chapter **5** the summary of the work and references are furnished.

Chapter 1

Cyanobacteria of Paddy fields

1.1 Introduction

The ability of algae to survive through 1.5 billion years was the consequence of wide spread compatibility and adaptability to the extremes of temperature, desiccation, illumination, radiations, salinity, pH, toxicants and nutrient availability. These attributes retained through the evolutionary sequences conferred cosmopolitan nature of algae especially cyanobacteria. The continuum helped them acquire attributes, which have far reaching implications in maintaining ecological balance and making agriculture sustainable.

In the cultivated fields algae occur even at 20cm depth with pronounced effect on the surface soil layer (Goyal, 1996). They have recently gained importance in agriculture as an input, which helps in better crop nutrient management (Goyal and Goyal, 1998). The ability of cyanobacteria to fix atmospheric dinitrogen is implicated in maintaining the

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spontaneous fertility of tropical rice field soil (Desikachary, 1959; Singh, 1961; Venkataraman, 1972, 1981; Goyal, 1993, 1995, 1996).

Microalgae as bioinoculants have been found to reduce the nitrogenous fertilizer consumption by about 30%. Whereas chemical fertilizers result in cyclic availability of nutrients, the microalgae provide biologically fixed nitrogen in a linear fashion. The amount of nitrogen contributed by cyanobacteria to rice crop varies from 20-30 kg/ha (Kaushik, 1994).

Singh (1961) reported *Aulosira fertilissima* as the dominating form in rice fields of India. He had reported that rice fields in higher elevations were dominated by members of *Oscillatoriaceae* while those at lower elevation contained a mixed population of *Oscillatoriaceae* and *Nostocaceae* members. Singh (1976) observed that species of *Aulosira, Wollea, Gleotrichia*, and *Anabaena* were mostly found in waterlogged rice fields.

Among the various factors that influence the distribution and abundance of blue-green algae in soil, pH is an important factor (Sardeshpande and Goyal, 1982). A decrease in light intensity and nitrogen level during the growth cycle of rice along with increase in pH level favoured cyanobacterial growth (Kanniayan, 1990). Numerous reports of cyanobacteria in freshwater and soil indicated that their diversity and abundance were greatest at higher pH (Kanniayan and Kumar, 2004) Development of algal bloom just after transplanting due to fertilizers or ploughing, or both, and a high light availability have been reported (Kanniayan, 1985). Begum *et al.* (1988) studied the effect of flooding on

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the qualitative and quantitative distribution of algae in deepwater rice fields and found that the growth of algal population increased by about 4-5 times during flooded period as compared to pre-flood and post-flood periods.

Kaushik (1999) reported that out of the five different species of cyanobacteria tested, *Nostoc* was the most predominant form followed by *Calothrix* when temperature varied from 10 to 35°C. In general, the temperature optima vary from 30 to 35°C.

Cyanobacteria make their presence conspicuous on the surface of rice field soil as well as flood water. Some of the common rice field cyanobacteria are *Anabaena, Aulosira, Calothrix, Gleotrichia, Cylindrospermum, Nostoc, Fischeiella, Scytonema, Tolypothrix, and Wollea* (Rai, 2001).

1.2 Enumeration of algal flora

In this investigation, the diversity of the algal flora of the unique low lying salinity prone paddy fields (pokkali fields) of Kochi, and that of an upland paddy field were studied. The low lying paddy fields are subject to salinity intrusion in summer and during this period the fields are used for prawn culture. In monsoon, when rainwater flushes away most of the salinity, the paddy variety 'pokkali' is sown. By tradition, pokkali cropping is just a one step exercise, which involves elaborate sowing procedures. The crop is not supported by any kind of manure or protected by any kind of pesticides. Therefore this ecosystem was selected for the investigation as it promises to offer a pristine environment. The upland paddy fields selected for the study were subject to agricultural practices such as manuring,

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weeding, and pesticide application. The diversity of the algal flora of these fields was studied in terms of the number and variety of the species.

1.2.1 Methodology

During the monsoon cropping season of 1999 and 2000, soil samples were collected from the pokkali fields and upland paddy fields for estimating the algal population. The cropping season began in late April and ended in early October. Soil sampling was done at a 15-day interval from the pre-sawing treatment stage of the crop. The low-lying saline fields were at Vytila, Ernakulam district (site-1). The upland fields chosen for the study were at Thrikkakara, Ernakulam district (site-2).

The algal population was assessed through MPN method (Pepper *et al.*, 1995). 10g of soil sample was suspended in 95ml of Bristol's medium and a serial dilution of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} was prepared. From each dilution, 1ml was transferred to 5 replicate tubes containing 9ml Bristol's medium. These tubes were incubated for 4 weeks on culture racks with 12/12 photoperiod, and the number of microalgae was enumerated. The biweekly enumeration results were clubbed to produce monthly reports.

To the end of incubation period of MPN study, microalgae from the least diluted tubes were harvested carefully through centrifugation, washed twice with distilled water, and photosynthetic pigments were extracted with acetone. The acetone soluble pigments were estimated spectrophotometrically following the method suggested by Becker (1994).

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Paddy field soil was inoculated to Schreiber's medium and BG11 medium; the algal growth formed was examined microscopically for the presence of cyanobacteria. Identification of the strains was done with the help of monograph of Desikachary (1959) and Anand (1989).

1.2.2 Observations

The paddy fields supported good growth of algae irrespective of the agricultural practices followed. In the saline fields the algal counts varied from 520 g^{-1} to infinite with chlorophyll a ranging from 0.216 µg l^{-1} to 31.411 µg l^{-1}. In the upland fields the algal flora ranged from 1070 g^{-1} in

Table 1.1: Number of microalgae estimated by MPN method in the pokkali fields of Vytila, Ernakulam district, during the period from April, 1999 to October,1999 and April, 2000 to October,2000 along with the chlorophyll a and chlorophyll b content obtained from MPN tubes of 10^{-2} dilution.

Period of sampling	MPN g^{-1}	Chlorophyll a µg l^{-1}	Chlorophyll b µg l^{-1}
April, 99	infinite	30.418	7.286
May, 99	infinite	28.290	6.912
June, 99	infinite	29.586	7.189
July, 99	infinite	31.411	6.834
August, 99	1474	0.581	0.101
Sept, 99	10305	4.895	1.018
Oct, 99	6329	3.215	0.928
April, 00	3036	1.682	0.429
May, 00	2957	1.595	0.312
June, 00	1570	0.856	0.178
July, 00	16509	6.541	2.008
August, 00	520	0.216	0.068
Sept, 00	1530	3.011	0.541
Oct, 00	5347	4.215	0.926

Table 1.2: Number of microalgae estimated by MPN method in the upland fields of Thrikkakkara, Ernakulam district, during the period from April, 1999 to October, 1999 and April, 2000 to October, 2000 along with the chlorophyll a and chlorophyll b contents obtained from MPN tubes of 10^{-2} dilution.

Period of sampling	MPN g^{-1}	Chlorophyll a $\mu g\ l^{-1}$	Chlorophyll b $\mu g\ l^{-1}$
April, 99	2196	1.532	0.258
May, 99	5876	3.519	0.582
June, 99	infinite	32.334	6.215
July, 99	infinite	30.512	6.185
August, 99	1584	1.386	0.232
Sept, 99	111203	11.295	2.351
Oct, 99	5340	3.051	0.521
April, 00	4306	2.816	0.489
May, 00	5425	3.015	0.511
June, 00	2270	1.586	0.291
July, 00	18608	6.093	1.018
August, 00	1070	1.002	0.261
Sept, 00	5650	5.326	0.395
Oct, 00	4506	3.886	0.268

August 2000 to infinite count in June-July 1999. The amount of chlorophyll a ranged from 1.002 $\mu g\ l^{-1}$ to 32.334 $\mu g\ l^{-1}$. There were no seasonal trends (Table 1.1 and 1.2). There was significant amount of chlorophyll b.

The population of cyanobacteria was quite diverse. The unicellular forms were represented by the genera *Aphanocapsa, Aphanothece, Chroococcus Gloeocapsa* and *Synechococcus;* the filamentous forms were represented by *Anabaena, Aulosira Cylindrospermum, Nostoc, Scytonema, Tolypothrix, Calothrix, Gloeotrichia, Westiellopsis, Microcoleus, Oscillatoria, Porphyrosiphon* and *Psudanabaena.* The nitrogen fixing

species observed were *Anabaena anomala, Anabaena fertillissima, Aulosira pseudoramosa, Cylindrospermum variabilis, Cylindrospermum muscicola, Cylindrospermum variabilis, Nostoc rivulare, Nostoc calcicola, Nostoc commune, Nostoc paludosum, Tolypothrix tenuis, Calothrix marchica* and *Gloeotrichia intermedia.* The non heterocystous filaments found in the fields were *Microcoleus lacustris, Oscillatoria earlei, Oscillatoria lutea, Oscillatoria margaritifera, Oscillatoria princes, Oscillatoria sancta, Porphyrosiphon notarisii, Phormidium stagnina* and *Psudanabaena constricta.* *Oscillatoria* was represented by the highest number of species followed by Nostoc and *Aphanothece.* The list of species encountered is presented below.

Synechococcus elongatus Nag.

Chroococcus minutus (Kutz.) Nag.

Gloeocapsa puntata Nag.

Aphanocapsa banaresensis Bharadwaja.

Aphanothece castagnei (Breb) Rabenh

Aphanothece microscopica Nag.

Aphanothece pallida (Kutz.) Rabenh.

Aphanothece stagnina (Spreng.)

Oscillatoria earlei Gardner.

Oscillatoria lutea Agardh. ex Gomont.

Oscillatoria margaritifera (Kuta.) Gomont.

Oscillatoria princeps var. pseudolimosa Ghose.

Oscillatoria sancta (Kutz.) Gomont.

Porphyrosiphon notarisii (Menegh.) kutz. Ex Gomont.

Phormidium stagnina Rao.

Microcoleus lacustris (Rabenh.) Farlow. ex Gomont.

Psudanabaena constricta (Szafer.) Lauterbom.

Cylindrospermum muscicola Kuta. Ex bom. et Flah.

Cylindrospermum stagnale Prasad.

Nostoc calcicola Brebisson. ex bom. et Flah.

Nostoc commune Vaucher. ex Bom. et Flah.

Nostoc paludosum Kutzing. ex Bom. et Flah.

Nostoc rivulare Kutzing. ex Bom. et Flah.

Anabaena anomala Fritsch.

Anabaena fertilissima Rao,C.B.

Aulosira pseudoramosa Bharadwaja.

Scytonema simplex Bharadwaja.

Calothrix marchica var. intermedia Rao.

Gloeotrichia intermedia (Lemm.) Geitler.

Westiellopsis prolifica Janet.

Tolypothrix tenuis Kuetz.

1. 3 Discussion

It was observed that the number of microalgae estimated during the monsoon-cropping season of 1999 and 2000, which were carried out at a two-week interval, did not show any regular pattern. The number varied from as low as 520 g^{-1} to infinite. The randomness of occurrence of microalgae was strikingly similar in both sites. All those positive and negative factors for algal growth generated at upland field might have been regenerated at the lowland field also due to the heavy water run off during monsoon.

Heavy monsoon and consequent flooding of the paddy field might disrupt the microalgal flora. Most of them might be washed off and the remaining might get diluted; the establishment of the flora has to start afresh every time. Also, rain water runoff produces turbidity. This might cut off light and reduce the number of suspended photosynthetic organisms. However, upon resumption of living conditions between heavy spells of rain, algae come back to existence with all its might. Repeated ebb and high in the number of algae during monsoon might therefore be due to mainly the rain activity and not due to any other biotic or abiotic factors. Algalization of paddy field during the period of monsoon therefore faces the challenge of inability of algal population density to remain consistent throughout the period of cultivation. Hence support from algae for paddy cultivation may become advisable only during the non-raining cropping season when the algal population does not get washed off.

The Cyanophycean population in the paddy field has shown a good diversity. Thirty one species of the genera *Synechococcus, Chroococcus, Gloeocapsa, Aphanocapsa, Aphanothece, Oscillatoria, Porphyrosiphon, Phormidium, Microcoleus, Psudanabaena, Cylindrospermum, Nostoc, Anabaena, Aulosira, Scytonema, Calothrix, Gloeotrichia, Westiellopsis* and *Tolypothrix* were obtained The findings corroborated with the earlier works (Anand and Hopper, 1987; Parukutty, 1940). However *Synechococcus elongatus* and *Tolypothrix tenuis* are reported for the first time from paddy fields of Kerala. Unicellular forms like *Synechococcus, Gleocapsa, Aphanocapsa, Aphanothece* were found to be present. Though not all of them are diazotrophic cyanobacteria, their role in the upkeep of the general soil health is to be reckoned with. Especially during the non-cropping

periods these microalgae actively interact with edaphic factors and establish an ecosystem of their own.

Though significance of *Oscillatoria* as N_2-fixing form is not well established, there are other filamentous forms encountered in the study, such as *Nostoc, Pseudanabaena, Anabaena, Scytonema, Gleotrichia, Westiellopsis,* and *Tolypothrix.* Their role in nitrogen fixation has been well established. However, use of nitrogenous fertilizer during the cropping season might repress the nitrogenase activity. Also, uncertainty in the population density throughout the period of cultivation make it a discontinuous source of nitrogen to the soil and hence unreliable.

The presence of diverse forms of cyanobacteria indicates a good balancing of the ecosystem. An unbalanced ecosystem or a polluted environment may have led to the reduction in the number of species and an increase in the number of individuals into a bloom.

Lowland rice fields particularly provide ideal environment for the luxuriant growth of blue-green algae (Singh, 2000). 41 species of filamentous forms from rice fields of Bhubaneswar belonging to the genera *Anabaena, Nostoc, Cylindrospermum, Calothrix, Microchate, Rivularia, Aulosira, Tolypothrix* and *Westiellopsis* were reported by Bastia *et al.* (1993). Mishra *et al.* (2001) have reported 30 nitrogen fixing heterocystous cyanobacteria from Terai belt of Uttar Pradesh. Ahmed (2001) recorded 9 genera of blue-green algae from Hojai subdivision of Assam of which only two – *Microcystis* and *Aphanocapsa* are unicellular forms; *Anabaena, Nostoc, Calothrix,* and *Aulosira* appeared to be most common blue-green

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algae in the sub division, followed by *Aphanocapsa* and *Microcystis*. Tiwari *et al.* (2001) report that the predominant members of filamentous cyanobacteria in rice fields of Uttar Pradesh are non-heterocystous types like, *Pseudanabaena, Limnothrix, Phormidium, Microcoleus, Oscillatoria, Lyngbya, Plectonema,* and *Porphyrosiphon*; whereas Singh (2001) claims that the algal flora of Rampur and adjoining area in Uttar Pradesh is dominated by hererocystous cyanobacteria.

A nation wide survey in India on nitrogen fixing algae have shown a predominance of *Nostoc* in the rice fields of Kerala, Tamil Nadu, West Bengal, Assam, and Haryana (Venkataraman, 1972). In Orissa, although *Nostoc* was dominant in the three agro climatic zones *Calothrix* was commonly encountered in most of the soils, followed by *Aulosira* and *Westiellopsis* (Sahu *et al.,* 1996). Amita Devi *et al.* (1999) have reported 110 blue-green algal forms belonging to 34 genera from rice fields of Manipur, where *Nostoc punctiformae* was the most dominant form, while *Anabaena variabilis, Calothrix marchia* and *Haplosiphon welwitshii* were fairly well distributed. Singh *et al.* (1996) have reported 17 species of *Chroococcales,* which is about 34% of the Cyanophycean population from rice fields of Mizoram. They have reported that *Aphanocapsa* dominated the algal flora followed by *Haplosiphon, Lyngbya, Anabaena, Nostoc* and *Cylyndrospermum,* in the descending order.

The present study indicates that the soil environment in the pokkali fields as well as the upland fields are conducive to algal growth, there are no exceptional variations from those reported from other parts of India.

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Chapter 2

Pesticide Tolerance of Cyanobacteria

2.1 Introduction

Improved high-yielding rice varieties, which are highly responsive to fertilizers, have become an integral part of cultivation practices. With higher fertilizer inputs used in intensive cultivation, disease and insect/pest problems have become prominent, necessitating the large-scale use of pesticides.

Though pesticides were the miracle drugs for the plants during 1950s, their hazardous effects on the environment were a matter of concern since the early 1960s. Despite improvement of crop plants through various breeding techniques, agriculture remains heavily dependent on these agrochemicals (Gadkari, 1988). The paddy field soil can be a natural sink for these pesticides, especially when recalcitrant forms are used.

Although studies on the interaction of cyanobacteria with agrochemicals continue to be widely conducted, there has been an increase in the amount of attention paid to pesticides, particularly insecticides. Insecticides, which constitute 83% of the total pesticides produce, have been studied quite extensively with respect to their effect on cyanobacteria. As a group, organochlorine insecticides are more hazardous to cyanobacteria due to their toxicity and persistence (Lal and Saxena, 1980). They have reported that organochlorines inhibit enzyme activity and photosynthesis; also interfere with the synthesis of DNA, RNA and proteins. Kar and Singh (1979) in a study on detoxification of HCH as (gammexane) by *Nostoc muscorum* and *Wollea bharadwajae,* found that 4 ppm HCH was algistatic to these two organisms. Sharma (1986) using cyanobacteria isolated from paddy fields found that maximum level of tolerance to lindane was 9ppm by *Anabaena doliolum,* 15ppm by *Aulosira fertilissima* and 10ppm by *Nostoc sp.* Tandon *et al.* (1988) observed an inhibitory effect of 1ppm endosulfan on an *Anabaena sp.* and also on *Aulosira fertillissima.* Sardeshpande and Goyal (1982) noted significant endosulfan-induced reduction in the growth of *Anabaena iyengarii, Haplosiphon intricatus* and *Calothrix bharadwajae.*

Carbamate group of insecticides have also been used for testing their influence on cyanobacteria. Kar and Sing (1979) have studied the effect of carbofuran (furadan 3%). Adhikary *et al.* (1984) found that sevin (50% w/v) at 10 μg ml^{-1} increased survival, growth and nitrogen fixation, while higher concentrations (>50 μg ml^{-1}) showed an inhibitory effect on *Anabeana sp.* and *Westiellopsis proligica.* Rath and Adhikary (1994) examined the relative tolerance in twenty two species of cyanobacteria to furadan

containing 3% active ingredient of carbofuran, and observed varied responses among different species and concluded that the ensheathed forms to be the ideal strains to be used as biofertilizer. Vaishmpayan (1984) reported that *Nostoc muscorum* was susceptible to carbaryl at 120ppm and it caused cell wall rupture and cell lysis.

Extensive research on the interaction of rice field cyanobacteria with organophosphate insecticides has been under taken in various laboratories of India because of their wide application in the tropical rice fields. These chemicals being biodegradable and non-persistent in the soil are popularized for pest management practices. Effect of organophosphate insecticide – malathion and phorate have been studied by Gangwane (1979), Sardeshpande and Goyal (1982), Kaushik and Venkataraman (1983) and Tandon *et al.* (1988). While studying the effect of monochrotophos and malathion on ten strains of filamentous heterocystous cyanobacteria, Subramanian *et al.* (1994) suggested that pesticides were used by these organisms as a sole source of phosphorous in the absence of inorganic phosphate in the medium and as an additional source of phosphorous when inorganic phosphorus was available in the medium.

Fungicides have potentially serious consequences on the overall productivity of soil, by interfering with the activity of cyanobacteria (Vyas, 1988). Venkataraman and Rajyalakshmi (1971) have found that the growth of *Cylindrospermum sp.* and *Nostoc muscorum* was inhibited at 1ppm and 5ppm of zinceb respectively in nitrogen deficient medium but tolerated higher concentrations in nitrogen supplemented medium. Soil fungicides

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nabam and vapan also showed varying degree of toxicity towards rice field cyanobacteria (Venkataraman and Rajyalakshmi, 1972); whereas captan at 1ppm level suppressed development, but not growth of *Nostoc muscorum.* Captan caused decrease in growth of *Westiellopsis prolifica, Aulosira fertillissima, Nostoc sp., Tolypothrix tenues,* and *Calothrix sp.* (Gangawane and Saler, 1979). Roval, a broad spectrum fungicide reduced growth of *Westiellopsis sp., Aulosira sp., Calothrix sp., Nostoc sp.,* and *Tolypothrix sp.* (Gangawane and Kulkarni, 1979). In general, fungicides reduce cell number, affect chlorophyll *a* content, decrease oxygen production, check CO_2 fixation, and cause increase in the proportion of carbon in sugar accompanied by reduction in lipids and glutamic acids.

Many of the herbicides used in paddy fields are known to inhibit photosynthesis. The growth and cellular nitrogen levels of *Anabaena doliolum* were reduced by uracil, isocil and terbacil (Kapoor and Sharma, 1980). In the same study, the benzoic acid herbicide was found to be slightly less toxic than uracils to *Anabaena doliolum.* The uracil isoproturon in the form of commercial preparation tolkan was tolerated by *Mastigocladus laminosus* and *Tolypothrix tenium* at concentrations 100 ppm and 500ppm respectively (Khalil *et al.,* 1980). Monuron, however was completely growth-inhibitory to *Nostoc muscorum* (Vaishampayan, 1984) at 0.34mM. The effect of the anilide herbicide propanil on *Nostoc muscorum* was found to depend on the size of the inoculum (Singh and Tiwari, 1988a). In another study Singh and Tiwari (1988b) have tested the effect of butachlor and fluchloralin on *Nostoc muscorum* and *Gleocapsa* species. Chinnaswamy and Patel (1983) observed the inhibition of growth of

Anabaena flosaquae by 10ppm and 25ppm basalin. The thiocarbamate benthiocarb, an inhibitor of protein synthesis, completely inhibited the growth and heterocyst differentiation in *Nostoc lincklia* at 4ppm; oxygen evolution was also reduced to one fourth of the control (Mishra and Pandey, 1989). Effect of 2,4-D on various species of cyanobacteria was studied by Mishra and Tiwari (1986) who observed an inhibition in the dry weight and nitrogen content and stimulation in chlorophyll *a* content in *Tolypothrix tenuis*. Venkataraman and Rajyalakshmi (1971) reported that the same species tolerated 200 kg ha^{-1} of 2,4-D under rice field conditions. This observation was further supported by Mishra and Tiwari (1986), who found that *Mastigocladus lamiaris* and *Tolypothrix tenuis* tolerated 5000ppm of 2,4-D.

In this study, the pesticides used extensively in the paddy fields of Kerala were screened against soil algae – cyanobacteria – to elicit, the tolerance level as well as residual effects on aquatic system. The test system used was *in vitro* cultures as well as soil microcosms.

2.2 Development of in vitro cultures

The standard methods employed in the isolation of cyanobacteria, (Stanier *et al.*, 1971) was followed in the present investigation, to develop pure cultures. Soil samples collected from paddy fields were inoculated into BG11 liquid medium. The blue-green growth formed were collected and streaked on to a BG11 solid medium (BG11 solidified by adding 1.5% Agar agar) in Petri dish. The colonies formed on the plate were examined microscopically and new culture was started by inoculating it on fresh

medium. The process was continued till an isolate was obtained in pure culture. The cultures were maintained in day-light fluorescent lamps at ambient condition (~30°C)

2.2.1 Characterization of the isolate – methodology
Cell Structure

The isolate obtained was examined under light microscope (LM). Further its smear was stained with nigrosin and air dried to examine the presence of capsule. Later the strain was viewed under the phase contrast microscope to examine the presence of sheath. The organism was also subjected to gram staining to verify its Gram positive or negative nature and to examine the external characters under stained condition. The size of the isolate was measured with micrometer.

The surface features of the isolate were studied further by Scanning Electron Microscopy (SEM) and sections viewed under Transmission Electron Microscope (TEM). The cells for Electron Microscope (EM) study were collected by centrifugation and repeatedly washed in distilled water. Washed cells were fixed in 2.5% glutaraldehyde and retained for 24 h at 4°C. The cells were centrifuged and washed for 10 min. in distilled water at 4°C. Washing was repeated, for 20 min. again at 4°C. The cells were transferred to phosphate buffer (0.2M, pH 7.2) and retained for 24 h at 4°C. These cells were post fixed in osmium tetroxide (1%) for 2 h (at 4°C). The fixative was washed off with phosphate buffer thrice. The fixed cells were suspended in 2% agar and the agar block was cut into bits. The embedded cells were dehydrated in a series of acetone at 4°C and brought to room temperature in absolute acetone. The cells suspended in acetone were dried

and sputter coated with gold and observed in scanning electron microscope. For transmission electron microscopy, the cells in acetone were transferred to Spurr's embedding medium. The embedded cells were cut into ultra thin sections using microtome. The sections were stained with uranil acetate and lead citrate (in 50% acetone) and observed under transmission electron microscope.

Absorption spectra

Photosynthetic pigments of the isolate were estimated following the method of Becker (1994). The cells harvested from the culture through centrifugation (3000 rpm, 10 min) were washed in distilled water and the pellet was ground in 100% acetone. The slurry was kept at 4°C in darkness for 24 h and then centrifuged at 3000 rpm for 10 minutes. The absorption spectrum was recorded in UV-VIS spectrophotometer.

Phycobilins were extracted in 0.15 M NaCl (Becker, 1994). Washed cells were suspended in 0.15M NaCl and repeatedly frozen and thawed. The cell suspension was kept at 4°C in darkness for 24 h for complete dissolution of phycobiliproteins. After centrifugation the supernatant was scanned in UV-VIS spectrophotometer.

Growth kinetics

Growth pattern of the isolate in BG 11 medium was monitored by cell count method. 250 ml medium taken in 500 ml sterile borosilicate culture flasks were inoculated with equal volumes of starter culture and incubated for 40 days under light assembly. The experiment was done in triplicate. Aliquots were drawn from the cultures on every alternate day and cells were

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counted directly in a haemocytometer. The divisions per day (k), during the exponential phase, were calculated according to Eppley and Strickland (1968) as follows: $k = 3.32/t_1-t_0$ ($\log 10 Nt_1$-$\log 10 Nt_0$); where, t_1-t_0 = time interval in days.

pH Tolerance

The isolate was grown in BG11 media adjusted to different pH in the range 4 to 10. The cultures were raised in 500 ml borosilicate culture flasks, inoculated with equal volumes of starter cultures, incubated under day light fluorescent lamps and yield was measured on the 15th day by estimating chlorophyll *a*.

Bioactive property

Sensitivity to Ampicillin: Test media were prepared by adding ampicillin to BG11 medium in concentrations 0.001, 0.01 0.1 and 1 mg l^{-1}. The new isolate was inoculated at 10^5 ml^{-1} cell density to these test solutions, in triplicate together with control. After incubation under light assembly for 96 h, the cultures were centrifuged and chlorophyll *a* was estimated to measure the growth response.

Antagonism to *Escherichia coli*: The standard disc diffusion method was employed to test antagonistic activity of the new isolate to *E. coli*. Filter paper discs (Whatman No. 1) of 5 mm diameter were dipped in aqueous, methanol, and ethanol extracts of the isolate and air-dried. These discs along with control disc were placed on an *E. coli* lawn culture on nutrient agar prepared in Petri plates. The plates were incubated in dark and

examined upon 12 h, 24 h, and 48 h for formation of clear zones around the discs.

Nitrogenase activity

Though most of the cyanobacteria do conduct nitrogen fixation, each isolated strain should be tested for its nitrogenase activity. It is probable that within a single species, there might be different ecotypes differing in their physiological characters.

An elementary study on N_2-fixing capability of *Synechococcus elongatus* was done by culturing them in BG11-N medium. Equal volumes of starter culture were inoculated to 200 ml of BG11- N and BG11+N medium, taken in 500 ml culture flasks. The experiment was done in triplicates and incubated under light assembly. The growth was assessed by measuring the chlorophyll *a* content of aliquots taken on alternate days.

The nitrogenase activity was assessed by acetylene reduction assay using gas chromatography (Taylor, 1983).

GC parameters

Detector: Flame ionization

Carrier gas: N_2 (30 ml min^{-1})

Detector gas: H_2 and air (30 ml min^{-1} and 300ml min^{-1} respectively)

Injection port temperature: 110°C

Oven temperature: 73° C

Detector temperature: 120° C

5 ml of dense culture (365×10^4 cells ml^{-1}) maintained in BG 11-N medium was taken in vials having 15 ml capacity. The vials were sealed with rubber septum and secured with aluminium cap using seal crimpers. 1 ml of the air (10% of the gas phase in the vial) was withdrawn from the vials, using a syringe and in its place 1ml acetylene was injected. The culture in the vials was incubated for 2 h under light (1500 lux) at $27 \pm 2°$ C. At the end of incubation 0.5 ml of 10% TCA was injected into the vials to arrest enzyme activity. Using a gas tight syringe 100 μl of gas was withdrawn from the vials and injected into the gas chromatograph. The amount of ethylene detected was expressed in μmol h^{-1} mg^{-1} of biomass. Biomass was calculated as chlorophyll-a μg ml^{-1} culture.

In order to find out the presence of nitrogenase activity in the anaerobic condition, one complete set of vials including the medium blank was flushed with argon to replace all the air in the gas phase of the vials. 1 ml of the gas was then withdrawn and 1 ml acetylene was injected into the vials. The culture in the anaerobic condition was incubated under light for 2 h and at the end of incubation, subjected to GC analysis.

Since the study material being a unicellular cyanobacterium there might be the possibility of a light-dark cycle of nitrogenase activity. Hence two complete set of vials, one each in aerobic and anaerobic conditions were incubated in dark for the same period. To the end of the incubation period the vials were subjected to ethylene assay.

2.2.2 Characterization of the isolate – Observations

The algal isolate obtained in pure culture was a unicellular cyanobacterium which has rod shape and usually appears as twin cells, with rounded tips. It is 5-6 μm long and 2-2.5 μm broad (Picture 2.1). The gram staining has shown typical red color indicating the gram negative nature. Upon negative staining with nigrosin, it showed no capsule. The phase contrast image had shown a thin sheath with lamellations. The sheath thickness increased with the age of the culture. The pure culture appeared bluish green to brownish-red in tint depending on the light intensity and density of culture. The strain reproduces by binary fission in a single plane, at right angle to the long axis of the cell. The strain is non-motile.

SEM studies have shown no special features on the surface of the organism (Picture 2.2). It appeared as smooth walled cells. The rod shape, rounded tip and 5x2.5 μm size were observed. The TEM studies have shown thylakoids and polyhedral bodies in thin sections (Picture 2.3). The organism was found to be having two electron dense cell wall layers and third layer lying in between them was electron transparent (Picture 2.4). Thylakoids form curved layers originating from the vicinity of the plasma membrane and arranged at approximately right angles to the cell wall. Many polymorphic bodies were located in the cytoplasm.

The 100% Acetone extract of the isolate yielded seven peaks of absorbance (Fig 2.1). Major peaks were at 665 nm and 430 nm.
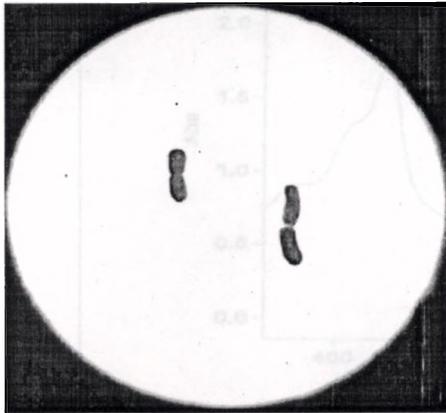
Picture2.1 Microphotograph of
Synechococcus elongatus (*400).
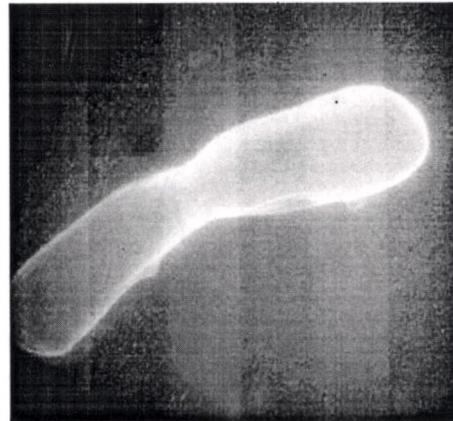
Picture2.2 SEM photograph of
Synechococcus elongatus (*5000).
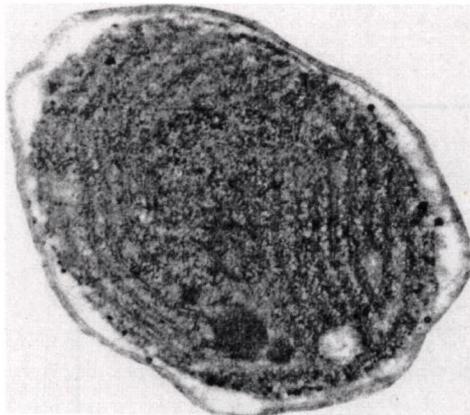
Picture2.3.TEM photograph of
Synechococcus elongatus upon
transverse section (*50000).
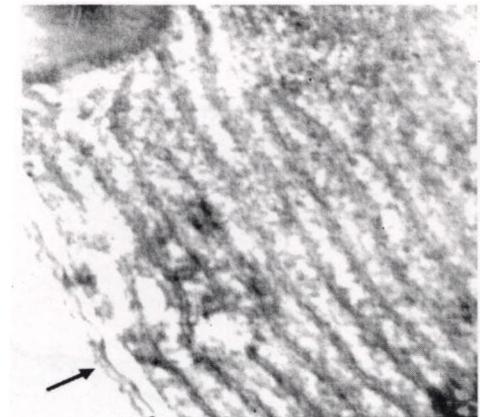
Picture2.4. TEM photograph of
Synechococcus elongatus
showing the wall layers (*80000).

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Fig 2.1: Absorption spectrum of acetone-soluble pigments of *S. elongatus*
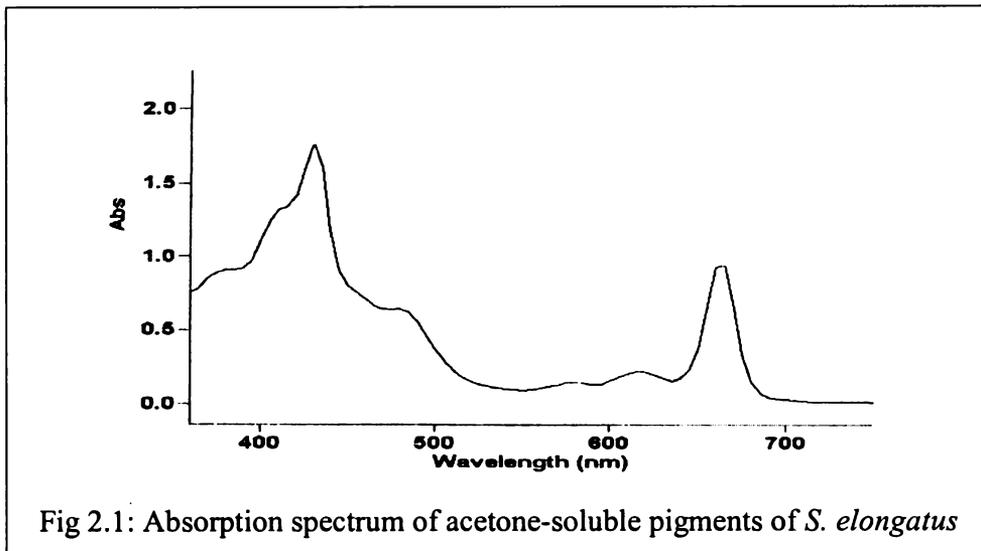
The extract of water-soluble pigments made in 0.15M NaCl revealed 2 major peaks of absorbance at 560nm and 620 nm (Fig 2.2).

Fig 2.2: Absorption spectrum of water-soluble pigments of *S.elongatus*
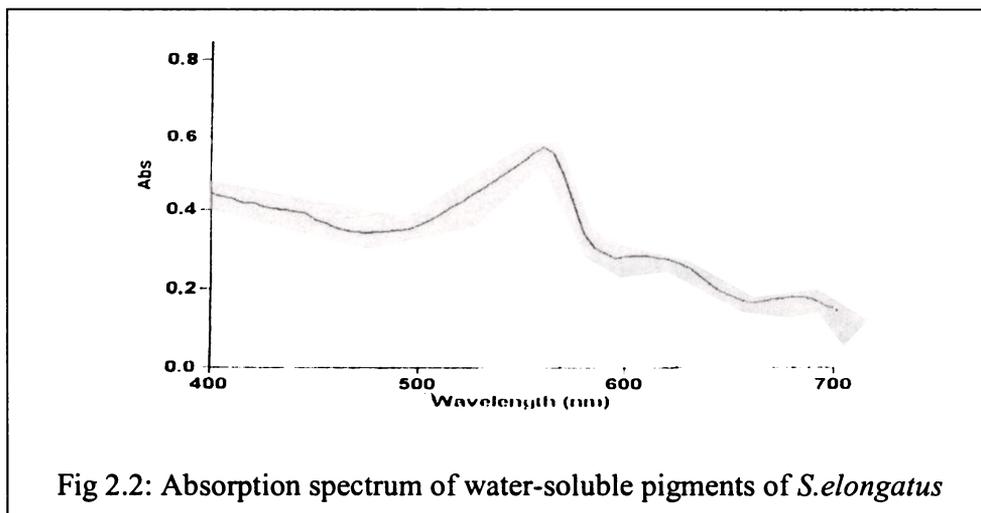
Growth kinetics study has shown growth pattern that conform with typical growth pattern of axenic cultures. The cultures reached stationary phase only by around 36nd day. The maximum divisions per day obtained was 0.14 and the population density attained a stationary phase was 309 * 10^4 cells ml^{-1} (Fig. 2.3).
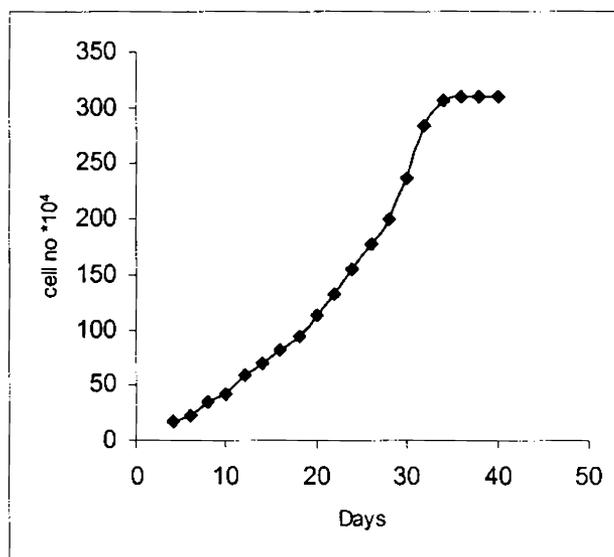
Figure 2.3 Growth curve of *Synechococcus elongatus* in BG11 medium

grown in day-light fluorescent lamp at ambient temperature

The growth of the cyanobacterium isolated was inhibited considerably below pH 6. The yield of chlorophyll *a* ranged from 30 to 260 µgl^{-1} in the pH range 4 to 10 (Fig 2.4). The analysis of variance of the yield in the pH range 6 to 9 was estimated to be insignificant (Table 2.1).

Table 2.1: Analysis of variance of the yield of cultures at pH 6 to 9

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	4706.3	3	1568.8	2.51	0.1325	4.0662
Within Groups	5000	8	625			
Total	9706.3	11				

Fig. 2.4: Amount of chlorophyll a ($\mu g \, l^{-1}$) yielded by *Synechococcus elongatus* in BG11 adjusted to different pH levels.
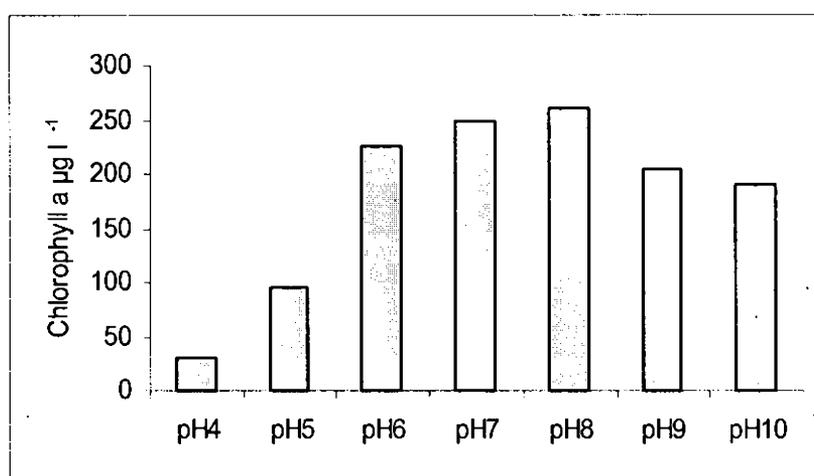
The newly isolated strain was highly sensitive to ampicillin. Whereas the control cultures yielded a biomass of 72.8 $\mu g \, l^{-1}$ of chlorophyll a following 96 h growth, the ampicillin test cultures did not show any measurable growth (Table 2.2)

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Table 2.2: Chlorophyll *a* yield of *S.elongatus* cultured in BG11 medium fortified with ampicillin.

Ampicillin mg l^{-1}	Chlorophyll *a* µg l^{-1}
Control	72.8
0.001	Not detectable
0.01	Not detectable
0.1	Not detectable
1	Not detectable

The strain did not exhibit bioactivity against *Escherichia coli*. Upon 12 h, 24 h and 48 h observations, the filter paper discs loaded with the extracts of the isolated strain, and kept upon *E. coli* lawn culture did not produce any clear zone around them.

The cyanobacterium showed the typical sigmoid curve of growth pattern in both nitrogen-free media and nitrogen-rich culture media. However the nitrogen-free cultures reached stationary phase on the 16th day, while, the culture in BG11+N attained stationary phase on 36th day. Also, the color of the nitrogen free culture eventually turned pale green from blue-green.
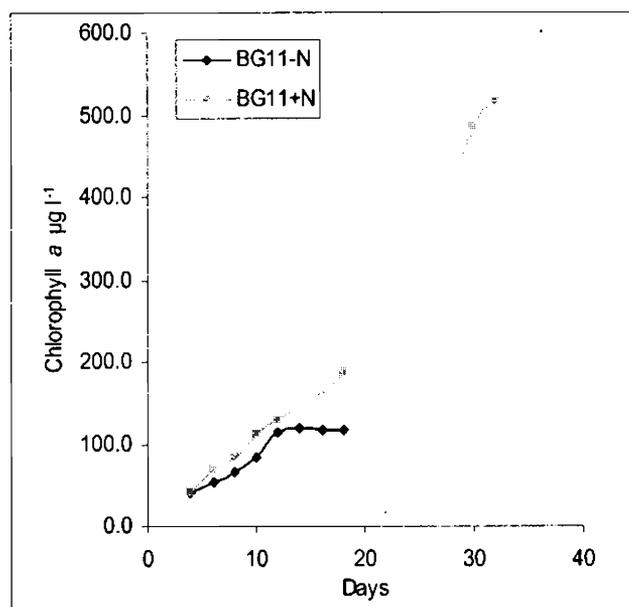
Figure 2.5: Growth curves of *Synechococcus elongatus* in BG11+N and BG11-N medium grown in day light fluorescent lamps at ambient temperature.

Acetylene reduction assay in the four different conditions – aerobic light, anaerobic light, aerobic dark and anaerobic dark conditions showed no detectable level of nitrogenase activity.

2.2.3 Identification of the isolate - discussion

The structural characters conform to the description of *Synechococcus elongatus*, as described by Desikachary (1959), Stanier (1971), Komarek (1976), Anand (1989) and Komarek (1999). Cyanobacteria that are single elongated erect cylindrical cells with transverse cell division, and without any firm vesicular sheath, appearing in group of 2-4 cells are grouped into genus *Synechococcus*. Those with cylindrical cells measuring 1.4 to 2 μm broad are identified as species *elongatus*. The LM and SEM results obtained

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with the present isolate strikes good congruence with the above description of *Synechococcus elongatus* Nag.

TEM studies have given a clear prokaryotic image of the strain. Smarda and Smajs (1999) have observed, in their freeze fracturing studies of pico-cyanobacteria of cell diameter up to 2 μm, that, exoplasm contains ribosome, 2-5 concentric parietal thylakoids, polyphosphate, and cyanophycin granules, and solitary vesicles. In sections, dense phycohilisomes were found attached to the thylakoid surface. Cell wall was 3 layered, with very poor mucilaginous sheath. Their next observation was that in *Synechococcus* binary fission takes place through cleavage and not through pinching off. The structure of the cell wall and nature of binary fission in the present study generally agree with the above reports. Also the presence of alpha phycpbilisomes on the thylakoid surface was very clear. The occurrences of solitary gas filled vesicles were also obvious in all the sections.

However, species level identification of cyanobacteria is a matter of great controversy. Many scientists prefer to circumvent the problem, and give strain numbers related to their laboratory. Graham and Wilcox (2000) advocated that cyanobacteria might be designated with culture collection numbers instead of identifying them to species. This may be taken as a pointer, that, isolates from different parts of the world may not easily fall in line with one Botanical description and therefore may be identified as different strains belonging to the same genus, and that, species level identification is irrelevant. Various culture collections of cyanobacteria have facilitated description of the taxa based on biochemical morphological

and genetic basis. Sequence data for 16S rDNA, DNA:DNA hybridization, and multi-gene sequence data are permitting rapid phylogenetically meaningful revision of large form genera like *Synechococcus* (Wood *et al.*, 2001).

The absorption spectrum of acetone extract of the isolate matched the characteristic curve of chlorophyll *a* with absorption maxima at 665 nm and 430 mn. The water-soluble pigments – phycobiliproteins showed an absorption maximum at 560 nm, characteristic of C-phycoerythrin. This may be treated as a mark of identity of cyanobacteria. Phycoerythrin from rhodophyceae is reported to have at least three absorption maxima, at about 498nm, 540nm and 565nm (Cohen-Bozire and Bryant, 1982). The second recognizable peak at 620 nm is the characteristic absorption maximum of C-Phycocyanin.

The maximum divisions per day attained by the culture was 0.14. Phillips *et al.* (1989) has reported a maximum growth rate of 1.28 divisions per day for *Synechococcus* species. The present strain is relatively slow growing, probably optimization of growth conditions could improve growth rate.

In pH tolerance studies, maximum output of chlorophyll *a* was obtained from cultures adjusted to pH 8 (250 $\mu g \, l^{-1}$), though an F-test indicates that there is statistically uniform yield from cultures adjusted to

pH6 to pH9. The cyanobacteria are reported to be capable of adapting to wide range of pH.

Synechococcus elongatus proved extremely sensitive to ampicillin; there was absolutely no growth even in the least concentration of ampicillin (0.001mgl^{-1}) provided in the medium. Stanier *et al.* (1971) has observed that most unicellular blue-green algae are extremely sensitive to penicillin G, and that they are similar to Gram +^{ve} eubacteria. Another reference of ampicillin in relation to cyanobacteria in the literature is that of Prabaharan *et al.* (1994), who reported that marine cyanobacterium, *Phormidium valderianum* BU30501 can make use of ampicillin as source of nitrogen, in the absence of combined nitrogen in the medium.

During the present study there was no evidence of production of bioactive compounds that were antagonistic to *Escherichia coli* by *Synechococcus elongatus*. But, many planktonic fresh water cyanobacteria possess antibacterial property (Ostensvik *et al.*, 1998). Methanol extract of *Tychonema bourrellyi, Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii* showed most pronounced inhibitory effects. Aqueous extracts from *mycrocystis aeruginosa* and *T. bourellyi* possessed evident antimicrobial properties. Cannell *et al.* (1988) after screening 100 cyanobacteria have reported that methanol extract of five show antibacterial activity against *Bacillus subtillis* and none against *E.coli*. In yet another study, Rao (1994) tested lipophilic extracts of 5 species of cyanobacteria from mangroves and found that extracts of *Anacystis dimidiate, Nostoc paludosum* and *Schizothrix sp.* were having antibacterial activity against

Bacillus subtilis. An antibiotic produced by *Nostoc linckia CALU 892*, named *Cyanobacterin LU -1* is reported to hinder cell division and light dependant oxygen evolution in *Synechococcus sp. R-2 (PCC.7942)* (Gromov *et al.*, 1991).

Synechococcus elongatus did not show any sign of nitrogen fixation in BG11-N cultures. Upon reaching the point of nitrogen starvation the strain started changing from blue-green to green. This is due to denaturation of phycobiliproteins, which act as accessory pigments as well as a nitrogen reserve (Lee, 1980).

Nitrogenase enzyme activity failed to be initiated in aerobic and anaerobic conditions as well as in light and dark incubation. Philips *et al.* (1989) has reported nitrogenase activity in unicellular *Synechococcus* sp. (designated BG0011), isolated from the costal lagoons of Florida. He has reported cyclic peaks in nitrogenase activity related to dark and light phases. The present isolate from the paddy field due to its niche in nitrogen excess environment would have lost its *nif* gene expression in the course of evolution. It is well accepted that nitrogenase activity initiates only in a nitrogen free condition. Nitrates have been found to inhibit nitrogen fixation in *Gloeothece* (Gallon, 1980) as well as in *Synechococcus* sp. (Huang and Chow, 1988). Addition of urea was found to inhibit nitrogenase in *Gloeothece* (Gallon, 1980). Therefore, years of adaptation to excess nitrogen condition might have forced this strain to shift to a non nitrogen fixing form by switching off the *nif* gene. A further study of the genome and

efforts to detect *nif* gene may be done to see whether this strain is genetically different from other *Synechococcus* strains reported earlier as nitrogen fixers

2.3 Algal tolerance of pesticides – in vitro studies.

The growth of *Synechococcus elongatus* upon exposure to 10 biocides were tested in laboratory batch cultures. The experiments were done in two steps – range finding tests and definitive tests. The biocides assayed are given in Table 2.3.

Table 2.3 Biocides assayed against in vitro cultures of *Synechococcus elongatus*

Trade name	Chemical name	Class	Chemical category
Nuvacron Classic Malathion	Monochrotophos Chlorpyriphos Malathion	Insecticide	Organophosphorus ,, ,,
Hilven	Fenvalarate		Pyrethroid
Captaf Zinthane	Captan Mancozeb Carbendazim	Fungicide	Phthalimide Dithiocarbamate Carbamate
2-4 D Roundup	2,4-D Glyphosate	Herbicide	Phenoxy alkanoic acid N-phosphonomethyl glycine
Bacterimycin	Bacterimycin	Bactericide	2-bromo 2-nitro propane 1,3-diol

2.3.1 Methodology

Range finding test

The cultures were inoculated at a cell density of 10^4 cells ml^{-1} to BG11 medium taken in borosilicate culture flasks, in triplicate, amended with pesticides at concentrations 0.01 mg l^{-1}, 0.1 mg l^{-1}, 1 mg l^{-1}, 10 mg l^{-1} and 100 mg l^{-1}. At the end of 96 h incubation (12/12light cycle), cells were harvested by centrifugation (3000 rpm, 20 min). The pellet was washed repeatedly in distilled water and ground in 100% acetone. The slurry was kept overnight under refrigeration for complete extraction of pigments. This was brought to room temperature under darkness and centrifuged to obtain chlorophyll extract. The optical density of the extract was measured for estimating chlorophyll a (Becker, 1994). On comparison with the control, percentage inhibition of growth was calculated.

Definitive test

Definitive tests were conducted in similar way as range finding test. The concentrations of pesticides were fixed based on the results of range finding test. Logarithmic scale concentrations were prepared by appropriately diluting stock solutions of pesticide (APHA, 1992). Out of the four insecticides studied, monochrotophos and chlorpyriphos were tested in concentrations 10 mg l^{-1}, 18 mg l^{-1}, 32 mg l^{-1}, 56 mg l^{-1} and 100 mg l^{-1} for definitive test on *Synechococcus elongatus*. Malathion was found to be more tolerated by *Synechococcus elongatus* and the test concentrations taken were 100 mg l^{-1}, 180 mg l^{-1}, 320 mg l^{-1} and 560 mg l^{-1}. The test concentrations of fenvalarate were 1 mg l^{-1}, 1.8 mg l^{-1}, 3.2 mg l^{-1}, 5.6 mg l^{-1} and 100 mg l^{-1}.

Of the three fungicides tested captan and mancozeb were taken in concentrations 10 mg l⁻¹, 18 mg l⁻¹, 32 mg l⁻¹, 56 mg l⁻¹ and 100 mg l⁻¹. Carbendazim was tested in concentrations 1 mg l⁻¹, 1.8 mg l⁻¹, 3.2 mg l⁻¹, 5.6 mg l⁻¹ and 10 mg l⁻¹.

The two herbicides tested were 2,4-D and glyphosate. Wherein, 2,4-D was tested in concentrations 320, 560, 1000 and 1800 mg l⁻¹, glyphosate was prepared in concentrations 1, 1.8, 3.2, 5.6 and 10 mg l⁻¹.

Bacterimycin – a synthetic bactericide, was found to be least tolerated in range finding test and therefore the concentrations tested in definitive test were 0.1, 0.18, 0.32, 0.56 and 1 mg l⁻¹.

After 96 h incubation, the total cell count was enumerated in haemocytometer and chlorophyll *a* was determined by spectrophotometer. Primary productivity was estimated by dissolved oxygen analysis (APHA, 1992). The results were compared with the control.

The pure cultures of *Chlorella pyrenoidosa* maintained in the laboratory of School of Environmental Studies were also assayed through the definitive test procedures followed for *Synechococcus elongatus*.

Statistical analysis

The data were analysed statistically to quantify and predict the probable effects of the pesticides on algae. The effect on growth of the various doses of pesticides were compared by ANOVA assuming the null hypothesis that all groups are equal *i.e.* the exposure to pesticides had no

effect on the organisms. Further Dunnet's analysis was done in cases where significant differences in treatment effect were observed, to identify the test concentrations that produced significantly lower/higher growth than control. The significant difference among the treatment dosages were brought out by the Tukey's multiple comparisons test. The data analysis was done with the software TOXSTAT. The dose effect relationship was computed by estimating the regression relation and the EC_{50} by probit analysis (software SPSS). The regression equation assumed that the amount of chlorophyll a to be linearly related to the pesticide dose. In the case of growth inhibition, the relation worked out is chlorophyll a = a-b * test concentration.

2.3.2 Results

Monochrotophos

Monochrotophos inhibited the growth of *Synechococcus elongatus*. There was about 50% reduction of growth as measured by chlorophyll a content of the cultures between the test concentrations of 10 mg l^{-1} and 100 mg l^{-1} of the insecticide (Table 2.4).

Table 2.4: Chlorophyll a (μg l^{-1}) of *Synechococcus elongatus* cultures amended with monochrotophos in a 96 h range finding test.

Sl.No.	Concentration mg l^{-1}	μg l^{-1} chlorophyll a		
		Mean	SD	% inhibition
1	0	67.920	0.412	0
2	0.01	57.793	0.712	15
3	0.1	52.907	1.597	22
4	1	46.047	1.261	32
5	10	37.013	1.569	45
6	100	28.453	2.591	58

The confirmatory test revealed that monochrotophos inhibits growth of *Synechococcus elongatus* significantly from 18 mg l^{-1} upwards (Table 2.5 and 2.6) as evinced by ANOVA and Dunnet's test. The cell count

Table 2.5: Results of definitive test on *Synechococcus elongatus* exposed monochrotophos

Sl. No.	concentration mg l^{-1}	Chlorophyll a μ gl^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity μg C $l^{-1}h^{-1}$	Net productivity μg C $l^{-1}h^{-1}$
1	0	47.50	14.20	46.11	38.60
2	10	45.60	13.65	42.80	35.80
3	18	40.70	12.18	39.50	33.80
4	32	38.10	11.39	36.20	30.20
5	56	32.00	09.57	29.60	24.80
6	100	20.20	06.05	19.70	16.50

Table 2.6: Toxicity study on *Synechococcus elongatus* with monochrotophos

SUMMARY STATISTICS					DUNNETTS TEST	TUKEY method of multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
1	control	3	47.500	0.656		6	\
2	10mgl^{-1}	3	45.600	0.964	2.295	5	* \
3	18mgl^{-1}	3	40.700	1.044	8.214 *	4	* * \
4	32mgl^{-1}	3	38.100	1.473	11.355 *	3	* * . \
5	56mgl^{-1}	3	32.000	0.656	18.723 *	2	* * * * \
6	100mgl^{-1}	3	20.200	1.058	32.977 *	1	* * * * . \

ANOVA	DUNNETTS TEST	TUKEY
F=295.106 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 1.028

Grp = Group; con = concentration; Chl a = Chlorophyll a μg l^{-1}

ranged from 14.2×10^4 to 6.1×10^4 cell ml^{-1} in the control and treatments. Gross productivity recorded was 46.11 µg /l/h to 19.7 µg C/l/h and net productivity was 38.60 µg C/l/h to 16.50 µg C/l/h. The Tukey method of multiple comparisons had brought out the significantly different groups (Table 2.6). The probit analysis results were EC_{50} 86.96 mg l^{-1} and regression equation y=3.476-1.793x.

The experiments with *C. pyrenoidosa* have shown significant level of growth inhibition from 10 mg l^{-1} onwards (Table 2.7 and 2.8). The test cultures have shown gross productivity of 54.9 µg C/l/h in control and 8.9 µg C/l/h in cultures amended with 5.6 mg l^{-1} monochrotophos. The corresponding net productivity were 43.8 µg C/l/h and 7.1 µg C/l/h (Table 2.7).

SI. No.	Concentra- tion mg l^{-1}	Chloroph yll *a* µg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	72.80	16.33	54.90	43.88
2	10	60.50	13.58	44.60	35.60
3	18	55.70	12.49	41.10	32.90
4	32	38.40	8.61	27.44	21.90
5	56	11.60	2.60	8.90	7.10

Table 2.7: Results of definitive test on *Chlorella pyrenoidosa* exposed to monochrotophos

F test carried out on chlorophyll *a* results gave F value of 433.556 rejecting the null hypothesis that all test groups are equal. The Dunnets test had shown that all test groups are significantly different from the control. The multiple comparisons done by Tukey method pointed out the significantly different groups (Table 2.8). Probit analysis gave EC_{50} 29.22 mg l^{-1} and regression equation y=3.822-2.617x.

Table 2.8: Toxicity study on *Chlorella pyrenoidosa* with monochrotophos

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY method of multiple comparisons	
Grp	con	N	MEAN Chl *a*	SD	T STAT	SIG	Grp	GROUP 0 0 0 0 0 / 5 4 3 2 1
1	Control	3	72.800	1.069			5	\
2	10mgl^{-1}	3	60.500	1.609	7.641	*	4	* \
3	18mgl^{-1}	3	55.667	0.318	10.643	*	3	* * \
4	32mgl^{-1}	3	38.400	0.603	21.370	*	2	* * . \
5	56mgl^{-1}	3	11.600	1.510	38.018	*	1	* * * * \

ANOVA	DUNNETTS TEST	TUKEY
F=433.556 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.65 s = 3.887

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l^{-1}

Chlorpyriphos

Range finding test using chlorpyriphos indicated an EC$_{50}$ beyond 10 mg l^{-1} and below 100 mg l^{-1} (Table 2.9).

Table2.9: Chlorophyll *a* (μg l^{-1}) of *S.elongatus* cultures amended with chlorpyriphos in a 96 h range finding test.

Sl.No.	Concentration mg l^{-1}	μg l^{-1} chlorophyll *a*		
		Mean	SD.	% inhibition/stimulation
1	0	53.500	0.656	0
2	1	64.767	1.484	21
3	10	47.367	1.124	12
4	100	9.467	0.513	72

The results of 96 h definitive test carried out with logarithmic concentrations of chlorpyriphos on *S.elongatus* are given in the Table 2.10. The control cultures produced an average of 72.9 µg l^{-1} chlorophyll *a* with a cell density of 17.8 x10^4 cell ml^{-1} The gross productivity registered was 57.8 µg C/l/h and net productivity 48.4 µg C/l/h. The yield was reduced upon pesticide exposure.

Table 2.10: Results of definitive test on *Synechococcus elongatus* exposed to chlorpyriphos				
Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* µg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity µg C l^{-1}h^{-1}	Net productivity µg C l^{-1}h^{-1}
1	0	72.90	17.80	57.80	48.40
2	10	69.40	16.90	54.90	46.40
3	18	57.00	13.90	4.50	37.20
4	32	25.50	6.50	20.80	18.30
5	56	12.20	2.90	9.20	7.70
6	100	8.10	1.90	6.30	5.80

ANOVA carried out on results of chlorophyll *a* estimation had given a F value of 814.701. This was followed by Dunnets test, which showed that all treatments groups except group 2 (*i.e.* 10 mg l^{-1}) were significantly different from the control group (Table 2.11). Probit analysis on results of chlorophyll *a* gave an EC$_{50}$ of 29.96 mg l^{-1} and regression equation y=4.461-3.029x.

Table 2.11: Toxicity study on *Synechococcus elongatus* with chlorpyriphos

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY method of multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 0	6 5 4 3 2 1
1	control	3	72.900	1.015		6	\	
2	10mgl^{-1}	3	69.400	3.365	2.437	5	. \	
3	18mgl^{-1}	3	57.033	1.872	11.048 *	4	* * \	
4	32mgl^{-1}	3	26.500	1.510	32.308 *	3	* * * \	
5	56mgl^{-1}	3	12.200	0.557	42.264 *	2	* * * * \	
6	100mgl^{-1}	3	8.133	0.351	45.096 *	1	* * * * . \	

ANOVA	DUNNETTS TEST	TUKEY
F = 814.701 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 3.094

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l^{-1}

Chlorella pyrenoidosa on a similar experiment with chlorpyrifos gave 74.9 μg l^{-1} chlorophyll *a* and 17 x 10^4 cells ml^{-1} in control cultures. The gross productivity and net productivity of control cultures were 54.9 μg C/l/hr and 46.6 μg C/l/hr respectively (Table 2.12). Growth was inhibited in the test cultures at different degrees depending upon the test concentrations.

Results of chlorophyll *a* upon analysis of variance gave a F value of 3614.963 against a critical value 3.48. The Dunnets test showed that all treatment groups were significantly different from control. The Tukey-

Table 2.12: Results of definitive test on *Chlorella pyrenoidosa* exposed to chlorpyriphos					
SI. No.	Concentration mg l^{-1}	Chlorophyll a µg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	74.90	17.00	54.90	46.60
2	10	23.90	5.44	17.40	14.80
3	18	13.20	2.99	9.30	7.90
4	32	10.10	2.29	7.10	6.30
5	56	7.30	1.66	5.10	4.30

multiple comparison had shown the test groups which are all significantly different from each other (Table 2.13). The EC_{50} was found to be 4.07 mg l^{-1} and regression y=0.569-1.103x upon probit analysis.

Table 2.13: Toxicity study on *Chlorella pyrenoidosa* with chlorpyriphos

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY method of Multiple comparisons					
Grp	con	N	MEAN Chl a	SD	T STAT	SIG	Grp	GROUP 0 5	0 4	0 3	0 2	0 1
1	control	3	74.900	0.436				\				
2	10mgl^{-1}	3	23.900	0.819	77.119	*	5	*	\			
3	18mgl^{-1}	3	13.200	0.872	93.299	*	4	*	*	\		
4	32mgl^{-1}	3	10.100	1.082	97.987	*	3	*	*	*.	\	
5	56mgl^{-1}	3	7.300	0.700	102.221	*	2	*	*	*	*	\
							1					

ANOVA	DUNNETTS TEST	TUKEY
F = 3614.963 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.65 s = 0.656

Grp = Group; con = concentration; Chl a = Chlorophyll a µg l^{-1}

Malathion

Malathion, another organophosphorus insecticide upon range finding test produced 76.06 µg l⁻¹ chlorophyll *a* in control cultures and 108.167 µg l⁻¹, 78.4 µg l⁻¹, 66.167 µg l⁻¹, and 58.0 µgl⁻¹ respectively (Table 2.14) in the succeeding test concentrations.

SI.No.	Concentration. mg l⁻¹	Mean	S.D.	%inhibition/stimulation
1	0	76.067	2.627	0
2	0.1	108.167	4.020	42
3	1	78.400	5.122	3
4	10	66.167	7.102	13
5	100	58.000	1.769	24

Table 2.14: Chlorophyll *a* (µg l⁻¹) of *S.elongatus* cultures amended with malathion in a 96 h range finding test.

Definitive test carried out using malathion and on *S.elongatus* gave about 50% growth inhibition above 180 mg l⁻¹. The degree of growth inhibition increased with test dose upto 560 mg l⁻¹(Table 2.15).

Table 2.15: Results of definitive test on *Synechococcus elongatus* exposed to malathion

SI. No.	Concentration mg l⁻¹	Chlorophyll *a* µg l⁻¹	Number of cells ml⁻¹ (x10⁴)	Gross productivity µg C l⁻¹h⁻¹	Net productivity µg C l⁻¹h⁻¹
1	0	64.10	18.10	58.70	49.20
2	100	50.50	14.26	45.60	38.20
3	180	36.70	10.38	32.60	27.30
4	320	15.50	4.38	13.40	10.95
5	560	7.50	2.13	6.50	5.50

Analysis of variance on results of chlorophyll *a* content showed significant difference among groups. The Dunnets test showed that all test groups are significantly different from the control. And Tukey method of multiple comparisons had revealed the effect all test doses to be significantly different (Table 2.16). A probit analysis have shown the EC_{50} 199.84 mg l^{-1} and regression equation y=1.680-2.272x.

Table 2.16: Toxicity study on *Synechococcus elongatus* with malathion

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons	
Grp	con	N	MEAN Chl *a*	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 5 4 3 2 1	
1	control	3	64.100	1.836		5	\	
2	100mgl⁻¹	3	50.500	2.007	11.381 *	4	* \	
3	180mgl⁻¹	3	36.700	1.229	22.929 *	3	* * \	
4	320mgl⁻¹	3	15.500	0.794	40.670 *	2	* * * \	
5	560mgl⁻¹	3	7.500	1.082	47.364 *	1	* * * * \	

ANOVA	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.75 s = 2.142
F=779.535 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l⁻¹

On comparison, *C.pyrenoidosa* produced less than 50% growth upon exposure to 100 mg l^{-1} malathion. These cultures gave 38.5μg l^{-1} chlorophyll *a* and 7.51 x 10^4 cells ml^{-1}. The gross productivity was 24.2μg/l/h and net productivity 19.1μgC/l/h (Table 2.17).

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Table 2.17: Results of definitive test on *Chlorella pyrenoidosa* exposed to malathion					
Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* µg l^{-1}	Cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	94.20	18.00	58.10	49.30
2	100	38.50	7.51	24.20	19.10
3	180	25.60	5.03	16.10	13.60
4	320	20.30	3.56	11.20	9.50
5	560	6.20	1.86	5.80	4.90

Dunnets test on the values showed that all test groups are significantly different from control. According to the Tukey analysis all test combinations were significantly different (Table 2.18). The EC_{50} upon probit analysis was 71.25 mg l^{-1} and regression equation was y=2.806-1.503x.

Table 2.18: Toxicity study on *Chlorella pyrenoidosa* with malathion

SUMMARY STATISTICS					DUNNETTS TEST	TUKEY multiple comparisons	
Grp	con N		MEAN Chl *a*	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 5 4 3 2 1
1	control 3		94.200	0.854		5	\
2	100mgl^{-1} 3		38.600	1.058	62.901 *	4	* \
3	180mgl^{-1} 3		25.700	1.400	77.495 *	3	* * \
4	320mgl^{-1} 3		20.400	1.058	83.491 *	2	* * * \
5	560mgl^{-1}		6.300	0.964	99.442 *	1	* * * * \
ANOVA					Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=14,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.75 s = 1.172	
F=2956.569 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal							

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* µg l^{-1}

Fenvalarate

Test doses of fenvalarate between 0.01 and 10 mg l^{-1} recorded reduced growth of *S.elongatus* (Table 2.19).

		μg l^{-1} chlorophyll *a*		
Sl.No.	Concentration mg l^{-1}	Mean	S.D.	%inhibition
1	0	64.700	0.400	0
2	0.01	64.100	0.557	1
3	0.1	58.800	0.361	9
4	1	42.533	0.252	34
5	10	12.100	0.100	81

Table 2.19: Chlorophyll a (μg l^{-1}) of *Synechococcus elongatus* cultures amended with fenvalarate in a 96 h range finding test.

The definitive test revealed that the cell density, chlorophyll *a* and productivity were reduced upon exposure to fenvalarate (Table 2.20).

Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* μg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity μg C l^{-1}h^{-1}	Net productivity μg C l^{-1}h^{-1}
1	0	53.40	16.20	52.60	44.80
2	1	49.20	14.87	49.30	40.60
3	1.8	45.80	13.91	46.50	39.20
4	3.2	40.40	12.27	39.40	34.10
5	5.6	30.10	9.01	29.50	25.20
6	10	10.40	3.16	13.15	8.40

Table 2.20: Results of definitive test on *Synechococcus elongatus* exposed to fenvalarate

The EC$_{50}$ was computed as 5.44 mg l^{-1}. The regression relation was y=1.680-2.272x. The Dunnets ANOVA test had shown that the growth in control medium was significantly higher than in other test groups. Multiple

comparisons had shown that there was no significant difference between the effect of test doses 1 and 1.8 mg l^{-1} (Table 2.21).

Table 2.21: Toxicity study on *Synechococcus elongatus* with fenvalarate

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons							
								GROUP						
Grp	con	N	MEAN Chl *a*	SD	T STAT	SIG	Grp	0 6	0 5	0 4	0 3	0 2	0 1	
1	control	3	53.400	2.402			6	\						
2	1 mgl^{-1}	3	49.200	1.400	2.643	*	5	*	\					
3	1.8mgl^{-1}	3	45.800	2.707	4.782	*	4	*	*	\				
4	3.2mgl^{-1}	3	40.400	1.929	8.181	*	3	*	*	*	\			
5	5.6mgl^{-1}	3	30.000	1.916	14.725	*	2	*	*	*	.	\		
6	10 mgl^{-1}	3	10.400	0.529	27.059	*	1	*	*	*	*		\	

ANOVA	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 3.788
F=198.741 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l^{-1}

Fenvalarate was more toxic to *C.pyrenoidosa* producing 50% growth inhibition at 2.82 mg l^{-1} (Table 2.22 and 2.23). The regression relation between dose and chlorophyll *a* production was y=1.054-2.238x. The Dunnets test showed that control was significantly different from all other test groups. And a multiple comparison showed significant differences among the test groups.

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Table 2..22: Results of definitive test on *Chlorella pyrenoidosa* exposed to fenvalarate					
SI. No.	Concentration mg l^{-1}	Chlorophyll a µg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg $l^{-1}h^{-1}$
1	0	71.20	15.02	48.48	41.14
2	1	71.60	15.08	48.57	41.38
3	1.8	44.60	9.39	29.04	.24.60
4	3.2	41.10	8.65	25.82	21.90
5	5.6	10.80	2.27	9.60	8.20
6	10	8.50	1.70	6.50	5.40

Toxicity 2.23: Toxicity study on *Chlorella pyrenoidosa* with fenvalarate

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY Multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0	5 4 3 2 1
1	control	3	71.200	0.794		5	\	
2	1 mgl⁻¹	3	44.600	1.868	30.620 *	4	. \	
3	1.8mgl⁻¹	3	41.100	0.624	34.649 *	3	* * \	
4	3.2mgl⁻¹	3	10.800	0.600	69.528 *	2	* * * \	
5	5.6mgl⁻¹	3	8.500	0.889	72.176 *	1	* * * * \	

ANOVA	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10)
F=1807.031 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal		= 4.65 s = 1.132

Grp = Group; con = concentration; Chl a = Chlorophyll a µg l^{-1}

Captan

The fungicide captan stimulated the growth of *S.elongatus* at lower doses, while higher concentrations >10 mgl^{-1} decreased growth (Table 2.24). Chlorophyll *a* production increased upto 64% when the growth medium was amended with 5 mg l^{-1} captan. As the concentration of captan was raised, there was suppression of growth.

Table 2.24: Chlorophyll *a* (μg l^{-1}) of *S.elongatus* cultures amended with captan in a 96 h range finding test.				
Sl.No.	Concentration mg l^{-1}	μg l^{-1} chlorophyll a		
		Mean	S.D.	%inhibition/stimulation
1	0	63.800	1.082	0
2	0.1	65.200	0.361	2
3	1	104.533	1.762	64
4	10	63.767	1.518	0
5	100	18.067	0.416	72

It was further confirmed (Table 2.25) at test dose > 100 mg l^{-1}, the production of chlorophyll *a*, cell density as well as productivity get reduced successively reaching < 50% of control at captan dose of > 56mg l^{-1}. The EC$_{50}$ was estimated to be 68.50 mg l^{-1}. The regression relation derived was y = 3.621 − 1.973 x.

Table 2.25: Results of definitive test on *Synechococcus elongatus* exposed to captan					
Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* μg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity μg C l^{-1}h^{-1}	Net productivity μg C l^{-1}h^{-1}
1	0	66.90	19.80	64.20	53.80
2	10	61.90	17.80	60.80	48.30
3	18	58.60	15.80	50.60	42.90
4	32	52.30	13.60	43.90	35.30
5	56	41.10	11.70	37.10	29.80
6	100	21.20	5.90	20.20	16.30

ANOVA on chlorophyll *a* contents and Dunnets test showed that test effects were significant. Tukey method of multiple comparisons showed that there was no significant variation between the dosages 10 and 18 mg l^{-1} of captan, while the difference at higher levels were quite significant (Table 2.26).

Table 2.26: Toxicity study on *Synechococcus elongatus* with captan

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT	SIG	Grp	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
1	control	3	66.900	1.015			6	\
2	10 mgl^{-1}	3	61.900	1.513	4.876	*	5	* \
3	18 mgl^{-1}	3	58.600	0.794	8.095	*	4	* * \
4	32 mgl^{-1}	3	52.300	1.229	14.239	*	3	* * * \
5	56 mgl^{-1}	3	41.100	1.513	25.162	*	2	* * * . \
6	100 mgl^{-1}	3	21.200	1.308	44.570	*	1	* * * * * \

ANOVA	Dunnett table value = 2..50	* = significant difference (p=0.05)
F=538.156 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal	1 Tailed Value, (P=0.05, df=12,5) Ho:Control<Treatmet	. = no significant difference Tukey value (6,12) = 4.75 s = 1.577

Grp = Group; con = concentration; Chl a = Chlorophyll a µg l^{-1}

Captan was highly toxic to *C.pyrenoidosa* as the growth and productivity were strongly inhibited at very low dose compared to *S.elongatus* (Table 2.27)

SI. No.	Concentration mg l^{-1}	Chlorophyll a µg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$

Table 2.27: Results of definitive test on *Chlorella pyrenoidosa* exposed to captan

SI. No.	Concentration mg l^{-1}	Chlorophyll a µg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	78.50	16.32	50.10	42.50
2	10	9.50	2.93	8.90	7.70
3	18	8.50	1.79	5.50	4.50
4	32	7.30	1.62	4.90	4.20

The effect of the captan dosages was significantly different from control (Table 2.28) and the intensity of growth did not increase

Table 2.28: Toxicity study on *Chlorella pyrenoidosa* with captan

SUMMARY STATISTICS					DUNNETTS TEST	TUKEY multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT SIG	Grp	GROUP 0 0 0 0 4 3 2 1
1	control	3	78.500	1.931		4	\
2	10 mgl^{-1}	3	9.500	1.082	71.627 *	3	.. \
3	18 mgl^{-1}	3	8.500	0.600	72.665 *	2	. . \
4	32 mgl^{-1}	3	7.300	0.557	73.910 *	1	* * * \

ANOVA	DUNNETTS TEST	TUKEY multiple comparisons
F=2646.861 Critical F value = 4.07 (0.05,3,8) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.42 1 Tailed Value, (P=0.05, df=8,3) Ho:Control<Treat met	* = significant difference (p=0.05) . = no significant difference Tukey value (4,8) = 4.53 s = 1.392

Grp = Group; con = concentration; Chl a = Chlorophyll a µg l^{-1}

significantly above the test concentration of 10 mg l^{-1} as recorded by the Tukey's analysis. EC_{50} was found to be 0.006 mg l^{-1} and regression equation was y=1.1420-0.841x.

Mancozeb

The fungicide mancozeb stimulated the growth of *S.elongatus* up to 1 mg l^{-1} of the application. At a test dose of 10 mg l^{-1} and above there was growth inhibition (Table 2.29).

		μg l^{-1} chlorophyll *a*		
Sl.No.	Concentration mg l^{-1}	Mean	S.D.	% inhibition/stimulation
1	0	53.500	1.637	0
2	1	64.767	0.987	21
3	10	47.333	0.814	11
4	100	9.500	0.200	82

Table 2.29: Chlorophyll *a* (μg l^{-1}) of *S.elongatus* cultures amended with mancozeb in a 96 h range finding test.

In the definitive test involving mancozeb at 10 ml^{-1} to 100 mg l^{-1}, successive reduction in growth and productivity were observed (Table 2.30). The test effects were significant (Table 2.31).

Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* μg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity μg C $l^{-1}h^{-1}$	Net productivity μg C l^{-1}h r^{-1}
1	0	56.40	15.80	51.30	42.90
2	10	46.00	12.90	41.80	32.50
3	18	32.90	9.05	29.20	24.40
4	32	22.10	6.21	19.40	16.20
5	56	13.80	3.87	12.30	10.30
6	100	10.40	2.91	8.10	7.10

Table 2.30: Results of definitive test on *Synechococcus elongatus* exposed to mancozeb

Probit analysis estimate of EC_{50} was 25.57 mg l^{-1} and regression equation as y=1.788-1.364x.

Table 2.31: Toxicity study on *Synechococcus elongatus* with mancozeb

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 0	6 5 4 3 2 1
1	control	3	56.400	0.656		6	\	
2	10 mgl^{-1}	3	46.000	1.442	12.354 *	5	* \	
3	18 mgl^{-1}	3	32.900	0.700	27.916 *	4	* * \	
4	32 mgl^{-1}	3	22.100	1.480	40.745 *	3	* * * \	
5	56 mgl^{-1}	3	13.800	0.265	50.604 *	2	* * * * \	
6	100 mgl^{-1}	3	10.400	1.058	54.643 *	1	* * * * * \	

ANOVA	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 1.063
F=942.589 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl a = Chlorophyll a μg l^{-1}

The inhibitory effect of the fungicide on algae was more pronounced in *C.pyrenoidosa*. The lowest test dose of mancozeb 10 mg l^{-1} itself was highly toxic as the cell division, productivity and chlorophyll *a* production were reduced drastically (Table 2.32). The EC_{50} was estimated to as 0.534 mg l^{-1}. The linear relation between chlorophyll *a* and test dose was y = 0.253 - .0739 x.

SI. No.	Concentration mg l^{-1}	Chlorophyll a µg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	82.90	18.20	58.10	49.30
2	10	13.60	2.68	8.30	7.40
3	18	9.50	2.16	6.70	5.60
4	32	6.90	1.79	5.80	4.80
5	56	5.30	1.48	4.80	4.60

Table 2.32: Results of definitive test on *C.pyrenoidosa* exposed to mancozeb

Dunnets analysis of variance showed that the treatment effects are significantly different, while above 32 mg l^{-1}, the effect is rather stationary (Table 2.33).

Table 2.33: Toxicity study on *Chlorella pyrenoidosa* with mancozeb

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT	SIG	Grp	GROUP 0 0 0 0 0 5 4 3 2 1
1	control	3	82.900	0.557			5	\
2	10 mgl^{-1}	3	13.600	1.044	97.875	*	4	. . \
3	18 mgl^{-1}	3	9.500	0.361	103.665	*	3	* * \
4	32 mgl^{-1}	3	6.900	1.480	107.337	*	2	* * * \
5	56 mgl^{-1}	3	5.300	0.200	109.597	*	1	* * * * \

ANOVA	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treat met	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.65 s = 0.752
F=4417.293 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl a = Chlorophyll a µg l^{-1}

Carbendazim

The results of the assay of *S.elongatus* against carbendazim showed that the fungicide was inhibitory to the organism at 1 mg l^{-1} (Table 2.34).

		µg l^{-1} chlorophyll *a*		
Sl.No.	Concentration mg l^{-1}	Mean	S.D.	% inhibition
1	0	58.067	0.153	0
2	1	52.467	0.666	10
3	10	17.167	0.252	70

Table 2.34: Chlorophyll *a* (µg l^{-1}) of *S.elongatus* cultures amended with carbendazim in a 96 h range finding test.

The 96 h definitive test carried out with logarithmic concentrations of carbendazim on *S.elongatus* showed successive inhibition of growth and productivity from test concentrations 1 to 10 mg l^{-1} (Table 2.35).

Table 2.35: Results of definitive test on *Synechococcus elongatus* exposed to carbendazim

Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* µg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	51.90	16.20	52.10	44.10
2	1	47.30	14.70	45.50	38.50
3	1.8	45.90	14.09	46.20	38.80
4	3.2	40.20	12.63	39.10	33.70
5	5.6	31.50	10.20	32.50	27.50
6	10	16.10	5.34	17.90	13.70

The test concentration 1 and 1.8 mg l^{-1} had the same effect upon statistical analysis. All other treatment effects were significantly different (Table 2.36). The EC_{50} was estimated to be 6.75 mg l^{-1}. The regression relation was y = 1.590 – 1.942 x.

Table 2.36: Toxicity study on *Synechococcus elongatus* with carbendazim

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY Multiple comparisons	
Grp	con	N	MEAN Chl *a*	SD	T STAT	SIG	Grp	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
1	control	3	51.900	0.346			6	\
2	1 mgl⁻¹	3	47.300	1.015	5.480	*	5	.* \
3	1.8 mgl⁻¹	3	45.500	0.608	7.624	*	4	* * \
4	3.2 mgl⁻¹	3	40.200	0.600	13.938	*	3	* * * \
5	5.6 mgl⁻¹	3	30.500	1.752	25.493	*	2	* * * . \
6	10 mgl⁻¹	3	16.100	1.179	42.647	*	1	* * * * * \

ANOVA	DUNNETTS TEST	TUKEY
F=496.466 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control< Treatmet	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 1.057

`Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l⁻¹

Table 2.37: Results of definitive test on *C. pyrenoidosa* exposed to carbendazim

Sl. No.	Concentration mg l⁻¹	Chlorophyll *a* μg l⁻¹	Number of cells ml⁻¹ (x10⁴)	Gross productivity μg C l⁻¹h⁻¹	Net productivity μg C l⁻¹h⁻¹
1	0	75.80	14.30	45.20	38.30
2	1	44.30	8.20	25.80	21.80
3	1.8	31.40	6.60	21.30	18.50
4	3.2	21.90	4.20	13.50	10.90
5	5.6	13.20	2.80	9.30	7.60
6	10	8.50	1.50	4.80	4.10

Chlorella pyrenoidosa under exposure to same concentrations of carbendazim produced similar results (Table 2.37 and 2.38); which upon

probit analysis gave EC_{50} as 1.32 mg l^{-1} and regression equation as y=0.182-1.449x.

Table 2.38: Toxicity study on *Chlorella pyrenoidosa* with carbendazim

SUMMARY STATISTICS					DUNNETTS TEST	TUKEY multiple comparisons	
Grp	con	N	MEAN Chl *a*	SD	T STAT SIG	GRP	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
1	control	3	75.800	0.794		6	\
2	1 mgl^{-1}	3	44.300	0.781	44.908 *	5	* \
3	1.8 mgl^{-1}	3	31.400	0.819	63.300 *	4	* * \
4	3.2 mgl^{-1}	3	21.900	0.608	76.843 *	3	* * * \
5	5.6 mgl^{-1}	3	13.200	1.179	89.247 *	2	* *. * * \
6	10 mgl^{-1}	3	8.500	0.872	95.947 *	1	.* * * * * \

ANOVA	DUNNETTS TEST	TUKEY multiple comparisons
F=2500.966 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 1.495

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l^{-1}

2,4-D

2,4-D, a growth regulator used widely as herbicide, stimulated the growth of *S. elongatus* at low test concentrations. There was 41% stimulation of growth at 1mg l^{-1} (Table 2.39). The rate of stimulation decreased towards 10 and 100 mg l^{-1} and beyond this test concentration

probably growth was inhibited as there occurred sharp fall in chlorophyll a at 1000 mg l^{-1}.

Table 2.39: Chlorophyll a ($\mu g\ l^{-1}$) of S.elongatus cultures amended with 2,4-D in a 96 h range finding test.				
		Mg l^{-1} chlorophyll a		
SI.No.	Concentration mg l^{-1}	Mean	S.D.	% inhibition/stimulation
1	0	62.133	0.351	0
2	0.1	72.100	1.480	16
3	1	87.400	1.044	41
4	10	71.700	0.361	15
5	100	64.800	0.656	4
6	1000	15.300	0.436	24

The results represented in Table 2.40 confirm the above observations. The growth and productivity are significantly reduced at 320 mg l^{-1}. The toxic effect seems to saturate at concentrations >1000 ml^{-1} as revealed by Tukeys comparison (Table 2.41). Probit analysis gave EC_{50} =440.39 mg l^{-1} and regression equation y=6.364-2.409x.

Table 2.40: Results of definitive test on Synechococcus elongatus exposed to 2,4-D					
SI. No.	Concentration mg l^{-1}	Chlorophyll $a\ \mu g\ l^{-1}$	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity $\mu g\ C\ l^{-1}h^{-1}$	Net productivity $\mu g\ C\ l^{-1}h^{-1}$
1	0	61.90	18.20	59.10	49.40
2	320	41.90	12.33	39.30	33.80
3	560	21.75	6.47	19.60	16.40
4	1000	8.50	2.52	8.20	6.80
5	1800	6.90	11.28	6.50	5.40

Table 2.41: Toxicity study on *Synechococcus elongatus* with 2,4-D

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons	
								GROUP 0 0 0 0 0 5 4 3 2 1
Grp	con	N	MEAN Chl *a*	SD	T STAT	SIG	Grp	
1	control	3	51.900	1.513			5	\
2	320mgl^{-1}	3	41.900	1.179	22.241	*	4	. \
3	560mgl^{-1}	3	21.757	0.850	44.629	*	3	* * \
4	1000mgl^{-1}	3	8.500	0.794	59.382	*	2	* * * \
5	1800mgl^{-1}	3	6.900	1.015	61.161	*	1	. * * * * \

ANOVA	DUNNETTS TEST (cont.)	TUKEY (cont.)
F=1364.312 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.65 s = 1.213

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l^{-1}

Reduction in growth occurred in *C.pyrenoidosa* upon exposure to 2,4-D at concentrations form 560 to 1800 mg l^{-1}. The treatment effects were statistically significant (Table 2.43). The EC$_{50}$ was 1354.86 mg l^{-1} and regression equation was y=7.332-2.365x.

Table 2.42: Results of definitive test on *Chlorella pyrenoidosa* exposed to 2,4-D

Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* μg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity μg C l^{-1}h^{-1}	Net productivity μg C l^{-1}h^{-1}
1	0	69.70	22.00	71.10	60.30
2	560	55.60	20.77	64.30	54.90
3	1000	41.40	17.78	58.10	49.30
4	1800	24.90	13.24	42.10	35.60
5	3200	18.50	7.90	25.80	21.90

Table 2.43: Toxicity study on *Chlorella pyrenoidosa* with 2,4-D

SUMMARY STATISTICS				DUNNETTS TEST	TUKEY multiple comparisons	
Grp	con N	MEAN Chl *a*	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 / 5 4 3 2 1
1	control 3	69.700	1.900		5	\
2	560mgl⁻¹ 3	55.600	1.609	13.534 *	4	* \
3	1000mgl⁻¹ 3	41.400	1.217	27.165 *	3	* * \
4	1800mgl⁻¹ 3	24.900	0.173	43.003 *	2	* * * \
5	3200mgl⁻¹ 3	18.500	0.656	49.146 *	1	* * * .* \

ANOVA	Dunnett table value = 2.47 1 Tailed Value, (P=0.05, df=10,4) Ho:Control<Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (5,10) = 4.65 s = 1.628
F=827.980 Critical F value = 3.48 (0.05,4,10) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* μg l⁻¹

Glyphosate

The addition of glyphosate to the growth medium of *S.elongatus* suppressed the growth of the species (Table 2.44).

Table 2.44: Chlorophyll *a* (μg l⁻¹) of *S.elongatus* cultures amended with glyphosate in a 96 h range finding test.				
		μg l⁻¹ chlorophyll *a*		
SI.No.	Concentration mg l⁻¹	Mean	S.D.	% inhibition
1	0	65.100	1.510	0
2	0.01	64.833	1.137	0
3	0.1	64.067	1.124	2
4	1	56.133	1.115	4
5	10	12.133	0.473	81

The confirmatory test results (Table 2.45 and 2.46) affirmed the high toxicity of the herbicide to the species. The test dose for 50% growth

SI. No.	Concentration mg l^{-1}	Chlorophyll a μg l^{-1}	Number of cells ml^{-1} ($\times 10^4$)	Gross productivity μg C $l^{-1}h^{-1}$	Net productivity μg C $l^{-1}h^{-1}$
1	0	71.30	16.30	52.92	44.30
2	1	66.10	15.11	49.60	41.50
3	1.8	62.20	14.21	46.30	38.70
4	3.2	59.20	13.53	42.90	35.90
5	5.6	18.70	4.27	13.20	11.70
6	10	9.80	2.23	7.20	5.50

Table 2.45: Results of definitive test on *Synechococcus elongatus* exposed to glyphosate

Table 2.46: Toxicity study on *Synechococcus elongatus* with glyphosate

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY multiple comparisons	
Grp	con	N	MEAN Chl a	SD	T STAT	SIG	Grp	GROUP 0 0 0 0 0 0 / 6 5 4 3 2 1
1	control	3	71.300	3.175			6	\ .
2	1 mgl^{-1}	3	66.200	2.166	3.571	*	5	.* \
3	1.8mgl^{-1}	3	62.200	0.600	6.371	*	4	* * \
4	3.2mgl^{-1}	3	59.200	0.794	8.472	*	3	* * . \
5	5.6mgl^{-1}	3	18.700	0.819	36.827	*	2	* * * . \
6	10mgl^{-1}	3	9.800	1.389	43.059	*	1	* * * * * \

ANOVA

F=689.976

Critical F value = 3.11
(0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Dunnett table value = 2.50
1 Tailed Value,
(P=0.05, df=12,5)
Ho:Control<Treatment

* = significant difference (p=0.05)
. = no significant difference
Tukey value (6,12)
= 4.75
s = 3.060

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inhibition (EC_{50}) was estimated to be 4.35mgl^{-1}. The linear regression relation was y=7.337-2.365x.

Table 2.47: Results of definitive test on *Chlorella pyrenoidosa* exposed to glyphosate					
SI. No.	Concentration mg l^{-1}	Chlorophyll *a* µg l^{-1}	cells ml^{-1} (x10^4)	Gross productivity µg C l^{-1}h^{-1}	Net productivity µg C l^{-1}h^{-1}
1	0	64.70	26.60	80.80	68.50
2	1	29.80	12.25	37.20	31.60
3	1.8	27.30	11.22	34.10	28.90
4	3.2	25.30	10.40	31.10	26.30
5	5.6	16.40	6.74	18.60	15.80
6	10	9.60	3.94	12.40	10.50

Table 2.48: Toxicity study on *Chlorella pyrenoidosa* with glyphosate

SUMMARY STATISTICS					DUNNETTS TEST	TUKEY multiple comparisons	
Grp	con	N	MEAN Chl *a*	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
1	control	3	64.700	0.781		6	\
2	1 mgl^{-1}	3	29.800	1.480	34.958 *	5	* \
3	1.8mgl^{-1}	3	27.300	0.755	37.462 *	4	.* * \
4	3.2mgl^{-1}	3	25.300	1.442	39.466 *	3	* * . \
5	5.6mgl^{-1}	3	16.400	1.609	48.381 *	2	* * * . \
6	10 mgl^{-1}	3	9.600	0.964	55.192 *.	1	* * * * * \

ANOVA	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control <Treatment	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 1.495
F=733.122 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* µg l^{-1}

The chlorophyll *a* content of *C.pyrenoidosa* was 29.8 µg l^{-1} at glyphosate concentration of 1 mg l^{-1} against a value of 64.70 µg l^{-1} in control. Similar result was obtained with cell count and productivity (Table 2.47, 2.48). The EC$_{50}$ was 1.01 mg l^{-1} and the regression of chlorophyll *a* on test dose was y=0.007-0.908x.

Bacterimycin

Bacterimycin (2-bromo 2-nitro propane 1,3-diol) is an antibacterial drug. A range finding test conducted gave an average of 81.6 µg l^{-1} chlorophyll *a* in control cultures. 0.01 mg l^{-1} to 10 mg l^{-1} bacterimycin in culture produced 98.8µg l^{-1} to 20.1µg l^{-1} chlorophyll *a* (Table 2.49).

		µg l^{-1} chlorophyll *a*		
Sl.No.	Concentration mg l^{-1}	Mean	S.D.	% inhibition/stimulation
1	0	81.600	0.700	0
2	0.01	98.867	0.929	21
3	0.1	62.233	0.451	24
4	1	40.433	1.079	50
5	10	20.133	0.814	75

Table 2.49: Chlorophyll-a (µg l-1) of *S.elongatus* cultures amended with bacterimycin in 96 h range finding test.

Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* µg l^{-1}	Number of cells ml^{-1} (x10^4)	Gross productivity µg C l^{-1}h^{-1}	Net productivity µg C l^{-1}h^{-1}
1	0	49.30	16.20	52.60	44.40
2	0.1	38.60	12.69	43.70	34.20
3	0.18	35.60	11.69	42.40	31.50
4	0.32	32.40	10.64	36.40	28.50
5	0.56	31.40	10.31	35.30	28.60
6	1	26.60	8.41	29.10	21.70

Table 2.50: Results of definitive test on *S.elongatus* exposed to bacterimycin

The concentrations of bacterimycin chosen for definitive test were between 0.1 mg l^{-1} to1 mg l^{-1}. There was successive growth reduction with increasing dosage. The highest dose tested 1 mg l^{-1} inhibited chlorophyll a by 45% (Table 2.50). Therefore the EC$_{50}$ was assumed to be at concentration above 1 mg l^{-1}. The test results were statistically significant (Table 2.51). The effect of the test dose 0.32 mg l^{-1} and 0.56 mg l^{-1} overlapped.

Table 2.51: Toxicity study on *Synechococcus elongatus* with bacterimycin

SUMMARY STATISTICS					DUNNETTS TEST		TUKEY Multiple comparisons	
GRP	con	N	MEAN Chl a	SD	T STAT SIG	Grp	GROUP 0 0 0 0 0 0 6 5 4 3 2 1	
1	control	3	49.300	0.954		6	\	
2	0.1mgl^{-1}	3	38.600	0.656	14.963 *	5	* \	
3	0.18mgl^{-1}	3	35.600	1.136	19.159 *	4	* . \	
4	0.32mgl^{-1}	3	32.400	0.700	23.634 *	3	* * * \	
5	0.56mgl^{-1}	3	31.400	0.819	25.032 *	2	* * * * \	
6	1 mgl^{-1}	3	25.600	0.889	33.143 *	1	* * * * * \	

ANOVA	Dunnett table value = 2.50	* = significant difference (p=0.05)
F=253.837 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal	1 Tailed Value, (P=0.05, df=12,5) Ho:Control<T reatmet	. = no significant difference Tukey value (6,12) = 4.75 s = 0.767

Grp = Group; con = concentration; Chl a = Chlorophyll a μg l^{-1}

All the test concentrations of bacterimycin in the range 0.1 to 1 mg l^{-1} decreased the growth of *C.pyrenoidosa* significantly over the control

(Table 2.52 and 2.53). EC_{50} was found to be 0.022 mgl^{-1} and regression equation, y= 1.420-0.841x, upon probit analysis.

Table 2.52: Results of definitive test on *Chlorella pyrenoidosa* exposed to bacterimycin					
Sl. No.	Concentration mg l^{-1}	Chlorophyll *a* µg l^{-1}	Number of cells ml^{-1} ($\times10^4$)	Gross productivity µg C $l^{-1}h^{-1}$	Net productivity µg C $l^{-1}h^{-1}$
1	0	71.0	15.71	48.40	41.10
2	0.1	20.4	4.58	13.80	11.70
3	0.18	13.6	3.95	12.20	11.20
4	0.32	11.0	2.74	8.30	7.60
5	0.56	9.8	2.71	6.40	5.40
6	1	4.4	0.88	3.80	2.60

Table 2.53: Toxicity study on *Chlorella pyrenoidosa* with bacterimycin

SUMMARY STATISTICS						DUNNETTS TEST	TUKEY multiple comparisons	
Grp	con	N	MEAN Chl *a*	SD		T STAT SIG	Grp	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
1	control	3	71.000	1.473			6	\
2	0.1mgl^{-1}	3	20.400	1.136		65.988 *	5	* \
3	0.18mgl^{-1}	3	13.600	0.624		74.855 *	4	* . \
4	0.32mgl^{-1}	3	11.700	0.529		77.333 *	3	* * . \
5	0.56mgl^{-1}	3	9.800	1.015		79.811 *	2	* * * * \
6	1 mgl^{-1}	3	4.400	0.361		86.853 *	1	* * * * * \

ANOVA	Dunnett table value = 2.50 1 Tailed Value, (P=0.05, df=12,5) Ho:Control <Treatmet	* = significant difference (p=0.05) . = no significant difference Tukey value (6,12) = 4.75 s = 0.882
F=2067.080 Critical F value = 3.11 (0.05,5,12) Since F > Critical F REJECT Ho:All groups equal		

Grp = Group; con = concentration; Chl *a* = Chlorophyll *a* µg l ^{1}

Table 2.54:   Computed EC_{50} for various biocides, based on algal assay.

Test material	EC_{50} mgl^{-1}	
	Synechococcus elongatus	Chlorella pyrenoidosa
Monochrotophos	86.96	29.22
Chlorpyriphos	29.96	4.07
Malathion	199.84	71.25
Fenvalarate	5.44	2.82
Captan	68.50	0.006
Mancozeb	25.57	0.534
Carbendazim	6.75	1.32
2,4-D	440.39	1354.86
Glyphosate	4.35	1.01
Bacterimycin	>1	0.022

2.3.5 Discussion

The organophosphorus pesticides monochrotophos, chlorpyriphos and malathion inhibited the growth of *S.elongatus* and *C.pyrenoidosa*. The degree of toxicity varied with the pesticides and species (Fig. 2.6). The toxicity of the three insecticides were in the order

Fig. 2.6: EC_{50} computed for three insecticides based on algal assay.
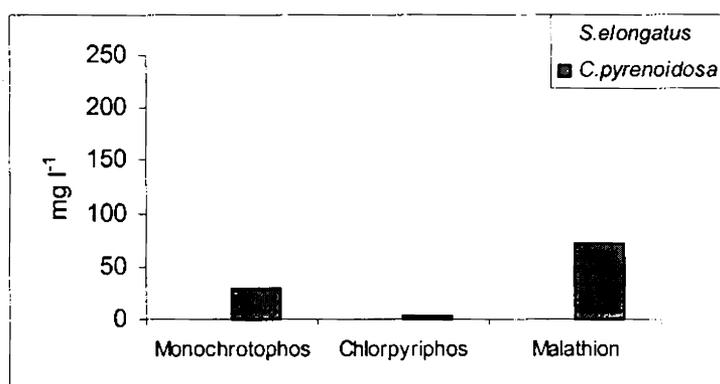
chlorpyriphos > monochrotophos > malathion and the sensitivity of the test species was *Chlorella pyrenoidosa* > *Synechococcus elongatus*. It was also observed that chlorpyriphos and malathion stimulated growth of the test species at low dose of ≤ 1 mg l^{-1}. It has been demonstrated earlier that the algae can utilize organophosphorus when given as the sole source of phosphate in the medium (Subramanian *et al.*, 1994; Nayak *et al.*, 1996).

The synthetic pyrethroid insecticide fenvalarate was inhibitory to the growth of the algae; the EC_{50} was lower than the organophosphorus pesticides, being 5.44 mg l^{-1} and 2.82 mg l^{-1} for *S.elongatus* and *C.pyrenoidosa*. The synthetic pyrethroids have been the recent replacement, mitigating evil hazards of other pesticides (Weston *et al.*, 2004). The observed high toxicity to non-target organism is a matter of concern.

Among the three fungicides tested, captan and mancozeb stimulated the growth of *S.elongatus* at doses upto 1 mg l^{-1}, where as carbendazim inhibited the growth of the test species by 10% at this test dose. The toxic effect of the three fungicides was evident at > 1 mg l^{-1} exposure. A revealing trend was the difference in the degree of response of the test species to the three fungicides. The toxicity to *S.elongatus* was carbendazim

> mancozeb > captan; while for *C.pyrenoidosa* it was captan > mancozeb > carbendazim. Obviously the toxicity is influenced by the property of the test species. The action of fungicides was reported to be drastic and acute on microalgae (Arton *et al.*, 1993, Anand and Subramanian, 1997).

The herbicide 2,4-D stimulated the growth of *S.elongatus* upon exposure upto 100 mg l^{-1}; further increase in dosage inhibited growth. Glyphosate, another herbicide tested was highly toxic to both the test species. It is quite natural that herbicides have harmful effects on another plant system. Piri and Oerdog (1999) have observed that herbicides are more toxic to green algae *Scenedesmus oblusiusculus* and cyanobacteria *Anabaena flosaquae*.

Bacterimycin, an antibacterial drug was observed to be highly toxic to *S.elongatus* and *C.pyrenoidosa* with EC50 >1 mgl^{-1} and 0.022 mgl^{-1}. The test strain of *S.elongatus* is very sensitive to ampicillin and therefore it is likely that this prokaryotic cyanobacterium is susceptible to antibiotic. Gromov *et al.* (1991) have reported that antibiotic cyanobacterin hinders cell division and light depended O$_2$ evolution in *Synechococcus* species. But the sensitivity of *C.pyrenoidosa* is noteworthy. This observation is against the normal view that prokaryotes are more sensitive to bactericidal drugs than eukaryotes.

The general picture given by the study is that *C.pyrenoidosa* is more sensitive to all biocides than *S.elongatus*, except 2,4-D. The only bactericide studied, bacterimycin was less toxic to the cyanobacterium. It was observed by Sabater and Carrasco (2001) that insecticides fenitrothion and

pyridaphenthion were less hazardous than the herbicides atrazine, benthiocarb cinosulfuron, chlorosulfuron, methyl-bensdulfuron and molinate to five fresh water phytoplanktons. The findings in the present study are in agreement with this as far as glyphosate is concerned. However, the other herbicide 2,4-D was well tolerated than most other biocides by both *S.elongatus* and *C.pyrenoidosa*. A similar result was reported by Nilima and Tiwari (2001) in *Anabaena variabilis,* which tolerated 260 mg l^{-1} 2,4-D, while aldrin and dimecron, were tolerated and 100 and 50 mg l^{-1} respectively, and endosulfan, malathion and parathion not above 25 mg l^{-1}. DeLorenzo *et al.* (2001) also reported that herbicides are generally more toxic to phototrophic microorganisms, exhibiting toxicity by disrupting photosynthesis. In another study, insecticide lindane was found to have drastic effect on biomass production and photosynthetic rate of cyanobacteria *Anabaena* (Suresh *et al.*, 2001). Wendt-Rasch *et al.* (2004) in their study on species composition of periphytic algae in *Elodea* dominated microcosm made an observation that the structure of ecosystem influences the final effect of pesticide exposure.

Chapter 3

Soil algalization – interaction with pesticides

3.1 Introduction

Repeated addition of pre-cultured microalgae – algalization - into agricultural soil can lead to the establishment of the algae with favorable modification of the physical and chemical conditions of the soil. Algalization has been promoted to increase the productivity of tropical paddy fields. Purposive and deliberate introduction of cyanophycean members reduce the nitrogenous fertilizer consumption. Substantial quantities of amino acids like alanine, aspartic acid and glutamic acid, vitamins like B_{12} and, auxin like substances are liberated by algae. These extra metabolites form a source of directly available nitrogen as well as accelerate crop growth enabling the crop plants to utilize more of the applied nutrients. Oxygen produced during algal photosynthesis reduces the oxidizable matter content in the soil. Algalization was shown to reduce iron toxicity by creating oxidizing conditions in the root zone of rice plants, which converted Fe^{++} to Fe^{+++} making it insoluble thereby reducing the iron

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content of the water (Goyal and Goyal, 1998). Growth of micro algae has a modifying effect on soil pH bringing it to almost neutrality from the range of 6.5-8.5 (Ansaveni and Kannaiyan, 1995). Algae have long been accredited as tools for reducing the salinity in the soil (Goyal, 1997). They create microenvironments in the root zone of rice plans with highly reduced salinity, which leads to better crop response.

Repeated algal application for 4-5 consecutive seasons ensures algal establishment, which sustains the algalization effect in the absence of fresh inoculation. The need of the hour is only to identify 'super' strains, performing the desired functions, grow them on large scale and make available quality inocula on demand. In view of the tremendous potential of algae, systematic survey of the autochthonous algae and their screening will provide the germplasm to choose from.

As algalization is a recommended practice of tropical paddy cultivation, so also pesticide application to control pests. The persistence of pesticides and its rates of entry into aquatic systems have been worked out in many instances (Ammato *et al.*, 1992). Mesocosm studies of pesticide use experimental ponds and *in situ* enclosures to which pesticides are applied (Toüart, 1988). Using this definition, small laboratory sized chambers and microorganisms used for studies may be referred to as microcosms (Nimmo and Mc Even; 1994). Use of microcosm appears to be excellent for controlling physical aspects of systems to study processes including pesticide degradation or transformation, acute effects or interactions of two species (Kersting and Van, 1992; Stay *et al.*, 1989; Lewis *et al.*, 1985; Pourtier, 1985 and Stay *et al.*, 1985). Therefore in the present study,

microcosm based studies were conducted to quantify pesticide residues and its leaching from the system. Pesticide residue was estimated by gas chromatography.

Several popular soil-applied herbicides can be commonly detected in surface water following run off events (Fawcett *et al.*, 1997). Leistra and Boesten (1989) have reported measurements of residues of pesticides in shallow and deep ground water – mainly some triaazine herbicides and their transformation products, ranging between 0.1µg l^{-1} and 0.5 µg l^{-1}. Hernandez *et al.* (1998), in their study on pesticide mobility in the soil had predicted that atrazine and metribuzin (herbicides) were probable lechers while organophosphorus pesticides (efnamiphos and chlorpyriphos) should be considered as improbable leachers. Pesticide leaching is inhibited by fine grained soil because of either low vertical permeability or high surface area; both enhance adsorption on the solid phase (Domagalski, 1992). However pesticides might leave the surface zone of soil by a variety of mechanisms (Yaron, 1989); they may leach downward with flowing water, volatilize to the atmosphere or chemically or biologically transform to new form. In an analysis of 270 ground water samples collected from the vicinity of rice fields, it was found that the mean concentrations of six commonly used pesticides ranged from 0.002 ppb for chlorpyriphos to 0.209 ppb for monochrotophos (Castaneda, 1996).

How do the rice field pesticide residues affect the soil algae? Does algalization alter the pesticide leaching from the fields? These questions were addressed through a soil microcosm study using *S.elongatus* as test organism.

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3.2 Methodology

3.2.1 Soil algalization

Synechococcus elongatus was inoculated into a soil microcosm, and its effect on soil properties was studied. Soil from paddy field was collected and filled into nine pots of similar size. Fertilizer (urea) was added to three of these pots and was inoculated with *Synechococcus elongatus* suspension in BG11 culture medium. Another set of three pots were directly inoculated with *Synechococcus elongatus* to serve as control for fertilizer effect. The remaining three pots were kept as blank controls. The experimental pots were incubated outdoor with regular watering for 15 days. The soil samples were analysed after incubation for the following attributes.

1) Soil pH: 20g of soil sample was shaken with 40ml distilled water for half an hour in a rotary shaker. The slurry was decanted and then filtered using Whatman No 1 filter paper and the pH of the filtrate was measured with glass electrode.

2) EC: The filtrate from the above was used to measure the electrical conductivity with the help of a conductivity meter.

3) Chlorophyll *a*: The amount of chlorophyll *a* in the soil was measured as an indication of total algal biomass. Chlorophyll *a* was extracted with 90% acetone (APHA, 1992) and estimated by spectrophotometry.

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3.2.2 Pesticide residue and algal growth

Paddy field soil was collected and filled into six similar sized pots. They were enriched with urea as in the previous experiment. Chlorpyriphos was added to all of these to a final concentration equivalent to $375g\ ha^{-1}$. To three of pots were inoculated with a suspension of *Synechococcus elongatus*. The initial concentration of chlorpyriphos was determined by gas chromatography. The soil microcosm was kept in day light with regular watering so that algal growth was promoted. The period of incubation was 15 days upon which the soil was sampled and analysed for chlorpyriphos. The soil sample was dried at room temperature and sieved through 1mm mesh. 15 g sieved soil sample was mixed with 0.3 g activated charcoal, 2 g floristil and 10 g anhydrous sodium sulfate.

Glass column 22 mm X 60 cm was clamped vertically at 2 places. A cotton plug was inserted at the bottom of the column with the help of a glass rod and 3 cm layer of anhydrous sodium sulfate was packed over it. Soil sample mixture was transferred into the column. After filling, the column was tapped gently to get a uniform packing. Pesticide from the column

GC parameters used.

Gas chromatography	Chemito 8610HT
Column	12 mm mega bore column with stationary phase BP-1 (100% Dimethylpolysitoxane film thickness 0.53 mm)
Column temperature	180° C
Detector	ECD

was eluted with 10% acetone in hexane (100 ml) and the elutant was collected drop wise in 4-5 h in the pear shaped 600 ml rotary evaporation flask. Finally the elutant was concentrated to about 1-2 ml. The final volume was made up to 10ml with hexane in a graduated stoppered test-tube and pesticide was quantified in gas chromatography

A similar set of experiment was carried out using monochrotophos; the concentration of monochrotophos addition was equivalent to 500 g ha^{-1} in the experimental pots.

3.2.3 Pesticide leaching and algal growth

The effect of pesticide leachates from soil was studied using laboratory microcosm. Paddy field soil was collected in similar sized pots and enriched with urea as in the previous experiments. Four pesticides were selected for the study. They were fenvalarate, malathion, 2,4-D and glyphosate. Each pesticide was applied to triplicate pots in concentrations equivalent to the EC$_{50}$ of each pesticide towards *S.elongatus* as reported in chapter 2. Another set amended with pesticides as above was algalized with *S.elongatus*. The rest of the samples free of pesticides but fortified with fertilizer were kept as control. After incubating the microcosms for 15 days, soil samples were collected, weighed and shaken with distilled water for half an hour in a rotary shaker. The filtrate was taken as leachate. This was amended with nutrients so as to attain the level of BG11 medium.

The leachates enriched with nutrients were inoculated with *S.elongatus* at a cell density of 2×10^5 cell ml^{-1}. After 96 h the cells were

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harvested by centrifugation. Chlorophyll *a* of the cell mass was estimated following the method of Becker (1994).

3.3 Results

3.3.1 Soil properties upon algalization

The soil supported the growth of *S.elongatus* upon enrichment with urea producing nearly three times biomass as measured by chlorophyll *a* (Table 3.1). The unfortified soil had only marginal growth of algae. In nitrogen enriched samples, the pH increased from 4.4 to 5.2. There was no significant change in electrical conductivity.

Table 3.1: Effect of algalization on the soil properties and algal biomass.

Soil attributes	Control	Algalized soil	Nitrogen enriched algalized soil
pH	4.48	4.91	5.21
EC (m mohs cm^{-1})	16.88	16.05	16.99
Chlorophyll *a* (μg g^{-1})	423.667±14.295	569.667±22.502	1228±93.402

3.3.2 Fate of pesticide upon algalization

The initial concentration of chlorpyriphos taken from control soil samples was 14.17 mg g^{-1} of dry soil. Upon algalization and incubation for 15 days the pesticide concentration was estimated as 13.57 mg g^{-1} of dry soil (Table 3.2). The pesticide concentration in the un-inoculated cultures was 13.63 mg g^{-1} of dry soil.

Table 3.2 Concentration of pesticide residues in paddy field soil amended
with chlorpyriphos

Category	Pesticide concentrations (mg g^{-1})	
	Initial	Final
Nitrogen enriched, pesticide amended soil	14.17 ± 0.960	13.63 ± 0.564
Nitrogen enriched, pesticide amended, algalized soil	14.17 ± 0.681	13.57 ± 0.775

In similar experiment using monochrotophos, the concentrations of pesticide in control soil was 18.94 mg g^{-1}. After 15 days of incubation the monochrotophos residue in non algalized soil samples was 18.18 mg g^{-1} and 17.28 mg g^{-1} in algalized soil samples (Table 3.3).

Table 3.3: Concentration of residues in paddy field soil fortified
with mochrotophos

Category	Pesticide concentrations (mg g^{-1})	
	Initial	Final
Nitrogen enriched, pesticide amended soil	18.94 ± 1.170	18.18 ± 0.285
Nitrogen enriched, pesticide amended, algalized soil	18.94 ± 0.550	17.28 ± 0.405

3.3.3 Leachate toxicity

The leachate from fenvalarate amended soil reduced the growth of *S.elongatus*. The biomass produced upon 96 h exposure was 125 µg l^{-1} and 99 µg l^{-1} chlorophyll *a* in non algalized and algalized soils. The respective control produced 232 µg l^{-1} chlorophyll *a* (Fig. 3.1).

Figure 3.1 :Chlorophyll *a* (µg l^{-1}) of *S.elongatus* cultured in leachate prepared from soil added with various pesticides
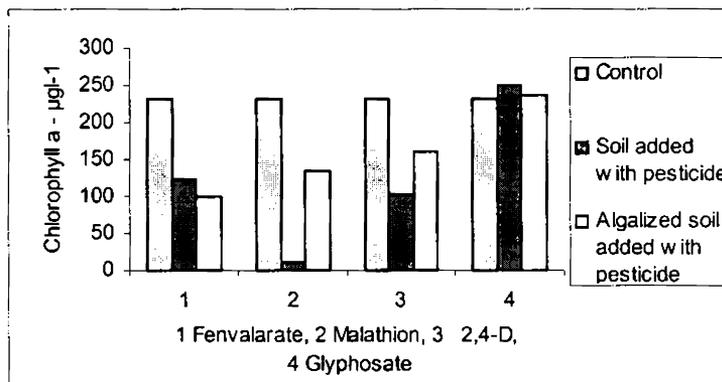
Malathion was added to soil at a concentration of 200 mg l^{-1} and the leachate gave 11 µg l^{-1} chlorophyll *a*, which was 95% growth inhibition compared to control; whereas leachate from algalized soil added with pesticide produced 135µg l^{-1} chlorophyll *a*.

Leachate from soil added with 2,4-D (450 mg l^{-1}) also showed inhibition of growth. Leachate from algalized, pesticide added soil microcosm gave 160 µg l^{-1} chlorophyll *a*, which was about 31% inhibition. And leachate from non-algalized, pesticide added soil gave 182µg l^{-1} chlorophyll *a*.

Microcosm experiment with glyphosate was done with 5 mg l^{-1} concentration. Leachate from the three test groups did not show any significant variation in the growth of *S elongatus* in them. Analysis of variance on the data gave F=1.916 against a critical value of 5.143 at 5% level of significance and 8 degrees of freedom.

3.4 Discussion
Algalization

Soil microalgae are recognized to have a vital role in the balancing of soil nutrients and making them available to the plants. Soil properties are found to be ameliorated to suit plant life by the soil flora. Algalization of soil at microcosm level with *S.elongatus* was monitored in the present study. Soil pH of the control soil was found to be 4.48. Growth of *S.elongatus* has increased the pH from 4.48 to 5.21. Addition of fertilizer has enhanced algal growth and hence further rise of pH. A good growth of unicellular alga can change acidic soil to neutral, making soil conditions more conducive for crops (Amsaveni and Kannaiyan, 1995). The F value obtained is 12.987 whereas critical value of F at 5% level of significance is 5.143. Therefore the differences in pH observed in the experiment were significant.

Conductivity is a measure of the total electrically charged ions in the system. The conductivity of control soil is 16.81 m mohs cm^{-1}, which has come down to 16.05 m mohs cm^{-1} in algalized soil and increased to 16.99 m mohs cm^{-1} in algalized-fertilized soil. It is evinced by the reading that algal growth may reduce conductivity by the absorption of mineral nutrients by the algae. However, on a later phase in the life cycle, these nutrients may be

secreted of excreted back to soil or may be returned by the death and decomposition of the algae; thus making the nutrients more readily absorbable and assimilable by the plants. Addition of urea in soil has increased the conductivity, which might take some more days of algal growth to consume all the fertilizer.

Chlorophyll a measurement has shown presence of microalgae in control soil as well. However the quantity of the natural flora is comparatively poor. Upon inoculation with $S.elongatus$ the chlorophyll a level has increased from 0.423mg l^{-1} to 0.569mg l^{-1}. Further increase of chlorophyll a to 1.228mg l^{-1} by addition of fertilizer in the soil could be observed which indicate that algalization with $S.elongatus$ becomes effective only upon addition of nitrogenous fertilizer.

Pesticide Residue

The nature and microbial degradation of organophosphorus insecticide by bacteria and fungi have been well documented. Many gaseous, solid and liquid recalcitrant pollutants including those of natural and xenobiotic origins $viz.$ carbon dioxide, nitrogen, phosphorus, phenolics, pesticides, antibiotics, lignin and detergents are detoxified or metabolized by cyanobacteria (Subramanian and Uma, 1999). Friensen-Pankratz et $al.$ (2003) observed that the presence of algae ($Selenastrum$ $capricornutum$) decreased the aqueous persistence of pesticides (atrazine and lindane), and speculated that algae either provided sites for pesticide sorption or facilitated pesticide degradation.

A specific report of cyanobacteria assimilation of organophosphorus pesticides as phosphorus nutrition has come from Subramanian *et al.* (1994). They have suggested the degradation of organophosphorus pesticides by acid phosphatase and subsequent metabolization, which was indicated by the enhancement of growth and other parameters in the absence of phosphate in the medium. There are earlier reports also that cyanobacteria assimilate phosphorus in excess of their requirements (Batterton and Van Baalen, 1968; Volk and Phynney, 1968; Stewart and Alexander, 1971). Healey (1982) had suggested that greater acid phosphatase activity and absence of detectable level of alkaline phosphatase in the presence of pesticides may be connected to the role of acid phosphatase in polyphosphate degradation.

However the present study shows residues of chlorpyriphos at a concentration of 13.63 mg l^{-1} of dry soil and 13.57 mg l^{-1} of dry soil after 15 days of algalization. Both have shown an initial concentration of 14.14 mg l^{-1}. Similarly, monochrotophos, another organophosphorus insecticide, has shown more or less same pattern of residue concentration; 18.18mg l^{-1} and 17.28mg l^{-1} in control and algalized soil respectively, and the initial concentration was 18.94 mg l^{-1}. It is evident that only a minor fraction has degraded in 15 days. One probability is that 15 days of incubation may not be just sufficient to enable algal degradation of organophosphates.

A second probability might be attributed to the number of extra cellular compounds released by the organism, which might interfere with the pesticides or their degraded products in direct gas chromatography (Subramanian *et al.*, 1994). Cyanobacteria are known to release a large number of extra cellular substances (Fogg, 1962; Whitton, 1965;

Subramanian and Shanmugasundaram, 1986), which might hinder with the monitoring of pesticide degradation.

Leachate toxicity

Leaching of pesticides from agricultural fields to nearby areas and even up to deep ground water table are being studied ardently. Leaching is one of the sure ways of pesticide transport in the soil. However many changes that might take place to a pesticide in the soil, might as well change it to a less toxic residue. An indirect method of evaluation the toxicity of residual molecules of pesticide from soil is adopted in the present study.

The soil leachates of fenvalarate, malathion and 2,4-D reduced the growth of *S.elongatus* when exposed to *in vitro* cultures. The test species was tolerant to glyphosate leachate. The toxicity of the leachates depends upon its water solubility as well as the chemical degradability and the toxicity of transformation products. In the present study the toxicity of the leachates upon algalization increased in the order fenvalarate > malathion > 2,4-D. The pattern of toxicity of leachates from non-algalized system deviated from the above, indicating interaction between algae, soil properties and pesticides.

The agricultural industry and urban pesticide uses are increasingly relying upon pyrethroid insecticides and shifting to more potent members of the class, yet a little information is available on residues of these substances in aquatic systems (Weston *et al.*, 2004). Fenvalarate (a pyrethroid) leachate from algalized microcosm was found to be more toxic than leachate from non-algalized system. An increase in toxicity of degradation products of the

pesticide was observed by Sinclair and Boxall (2003) as well in many cases they have studied; and they have put forward an explanation that the phenomenon may be due to either (1) presence of pesticide toxicophore in the degraded products; (2) the product may be the active part of the pesticide; (3) the product is accumulated to greater extend than parent compound; or (4) the product has a more potent mode of action than the parent. Yet another possibility is that algalization facilitates the leaching of the added pesticides and hence becoming more available in the leachate.

Leachate from algalized soil fortified with malathion was found to be remarkably less toxic than leachate form non-algalized soil. Evidently the organophosphate was degraded by *S.elongatus* into nontoxic products or utilized the same for metabolic consumption. In the previous study (Chapter 2) *S.elongatus* had shown a higher level of malathion tolerance and gave the highest EC_{50} of all insecticides (EC_{50} 199.84 mg l^{-1}). Moostafa and Helling (2001) observed a 25% faster degradation of isoproturon by cyanobacteria *(Anabaena)* than *Chlorella*. A similar case of pesticide detoxification was reported by Hoagland *et al.* (2002) in which metabolites of atrazine *viz.* deethylatrazine and deisopropylatrizine were found to be 16 to more than 300 times less toxic to diatoms and chlorophyte taxa. Leachate from the 2,4-D microcosm showed a similar detoxicfication by *S.elongatus*. However the difference in toxicity was not as vivid as that of malathion microcosm.

Leachate from glyphosate microcosm did not show any significant difference from that of control. Huang *et al.* (2004) reported that very small amount of glyphosate molecules were mobilized and only its transformed products were detected in their study. It seems in the present study that

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glyphosate molecules are adhered to the soil particles and are not available for algal toxicity or leaching. Hence the leachate from pesticide added soil and pesticide added algalized soil had produced algal growth equivalent to that of control.

Chapter 4

Concerted stress from pesticide and salinity in paddy field

4.1 Introduction

Salination of agricultural lands is considered to be a grave problem that limits crop yields. Over 950 million hectares, including nearly one third of total irrigated land amounting to 76.7 million hectares are estimated to be affected by salinity (Kaushik, 1998). In India alone, over 7 million hectares of arable land are inflicted by salinity. In Kerala, 26400 ha of paddy field are prone to salinity (Sasidharan *et al.*, 2002). Sodium is the chief cation responsible for the deterioration of soil properties upon salination. Studies relating cyanobacteria and salinity stress are considerably less in number. Cyanobacteria have been recommended for reclamation of the saline and alkali soils (Singh, 1961; Kaushik and Ummat, 1992; Kaushik, 1995). A major information is that Na^+ is essential for cyanobacterial nitrogenase activity (Apte and Thomas, 1980a, b, 1984, 1985, 1986; Apte *et al.* 1987; Thomas *et al.* 1988).

Many cyanobacteria exhibit considerable tolerance to salt and occurrence of marine, salt lake inhabiting or brackish water forms is not rare (Desikachary, 1959; Fogg, 1973). There are also well documented cases of halophilic cyanobacteria, such as *Microcolius* (Van Baalen, 1962), *Spirulina subsalsa* (Fogg, 1973), *Aphanocapsa halophylica* (Yopp *et al.*, 1978) and *Calothrix scopularum* (Stewart 1964; Tel-OR, 1980) and euryhaline cyanobacteria, capable of growing in fresh water as well as saline habitats (Richardson *et al.* 1983; Reed and Stewart, 1983).

During salt stress, plants and microorganisms resort to osmotic adjustments by building up high internal concentration of inorganic and/or organic solutes (Flowers *et al.* 1977, Szalay and MacDonald, 1980). In plants inorganic ions are sequestered in vacuoles, which occupy about 90% of the mature cell volume. Another mechanism of sodium tolerance is the highly selective transport of sodium in higher plants (Epstein, 1980), algae and bacteria (Szalay and MacDonald, 1980).

Freshwater cyanobacterium *Synechococcus elongatus* was studied even earlier in connection with salinity stress (Ray *et al.*, 1986). The isolate in the present study – *S.elongatus* was subjected to different salinity levels by amending BG11 medium with appropriate quantity of sodium chloride.

A survey of literature has shown that, sodium influx in the cells can lead to toxicity by itself, however a study on any possible magnification or reduction in the pesticide toxicity by the interference of salinity was found to be non-existent. Salinity being a feature of many low lying paddy fields

especially pokkali fields of the present study, it was thought befitting to peep into the interference of salinity in pesticide toxicity.

4.2 Methodology

4.2.1 Effect of salinity on *Synechococcus elongatus*

BG11 medium was amended with appropriate quantity of sodium chloride to obtain salinity of 5×10^{-3}, 10×10^{-3} and 15×10^{-3}. The media (100ml) were inoculated with equal volume of exponential phase cultures of *Synechococcus elongatus*. The experiment was carried out in triplicates under laboratory conditions as in previous experiments. The control cultures were raised in standard BG11 medium. The experimental set up was incubated under light assembly for 15 days.

The pH of the culture was measured initially and on the 4th, 10th and 15th day of inoculation. The biomass of the cultures was estimated as chlorophyll *a* on the 15th day.

4.2.2 Salinity and pesticide interference on growth of *S.elongatus*

The salinity-pesticide interference test was carried out on 96h basis by measuring the chlorophyll *a* output of the experimental cultures at twelve different combinations of levels salinity and pesticides. The levels of salinity tested were 5×10^{-3}, 10×10^{-3} and 15×10^{-3}. Two levels of pesticides were selected to elucidate the interference with salinity. These dosages of pesticides were equivalent to the respective concentrations estimated to inhibit 50% and 25% growth of *S.elongatus* as observed in the experiment 2.2. Simultaneous cultures were raised to serve as blank controls for salinity as well as pesticides. The pesticides used were chlorpyriphos (30 mg l^{-1} and

15 mg l^{-1}), monochrotophos (90 mg l^{-1} and 45 mg l^{-1}), fenvalarate (5 mg l^{-1} and 2.5 mg l^{-1}), malathion (200 mg l^{-1} and 100 mg l^{-1}), 2,4-D (450 mg l^{-1} and 225 mg l^{-1}), and glyphosate (5 mg l^{-1} and 2.5 mg l^{-1}. The experimental lay out is given below.

Pesticide mg l^{-1}	Salinity x10^{-3}			
	Nil	5	10	15
Nil	r_1 r_2 r_3	r_1 r_2 r_3	r_1 r_2 r_3	r_1 r_2 r_3
Level 1	r_1 r_2 r_3	r_1 r_2 r_3	r_1 r_2 r_3	r_1 r_2 r_3
Level 2	r_1 r_2 r_3	r_1 r_2 r_3	r_1 r_2 r_3	r_1 r_2 r_3

r_1, r_2, r_3 - replications

Proceedure : The BG11 medium amended with sodium chloride at various levels and added with respective levels of pesticides were taken in 250ml borosilicate culture flasks. They were inoculated with equal volumes of exponentially growing cultures of *S.elongatus*. After incubation for 96 h under day light fluorescence lamps, the cultures were centrifuged and chlorophyll *a* was determined. The results obtained were analysed by factorial analysis to elucidate the interference of salinity with pesticide activity.

4.3 Results

4.3.1 Effect of salinity on the growth of *S.elongatus*

The salinity of the growth medium was observed to reduce the growth of *S.elongatus*. The yield of chlorophyll *a* in the control was 128.6 $\mu g\, l^{-1}$. While at a salinity of 5×10^{-3}, the chlorophyll *a* content was 84.4 $\mu g\, l^{-1}$. The

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increase in salinity further decreased the chlorophyll *a* yield (Figure 4.1). Reduction of growth beyond 50% occurred at salinity $>10 \times 10^{-3}$.

Figure 4.1 Chlorophyll a yield of *S.elongatus* cultured in BG11 media amended to different levels of salinity.
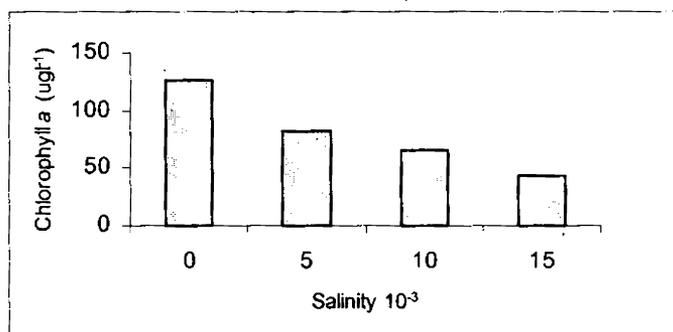
The pH of the growth medium increased over the days of growth irrespective of the salinity (Table 4.1)

Table 4.1 Change in pH upon culturing *S.elongatus* in BG11 modified to different salinity

Salinity $x10^{-3}$	Day 0	Day 4	Day 10	Day 15
Control	7.7	8.0	8.9	9.2
5	7.6	8.5	9.1	9.0
10	7.8	8.2	9.0	9.1
15	7.9	8.1	8.8	8.9

4.3.2 Salinity and pesticide interference on S.*elongatus*

Chlorpyriphos

Chlorpyriphos reduced the growth of *S.elongatus* by 23.2% when applied at a concentration of 15 mg l^{-1}. At 30 mg l^{-1} of the pesticide, the biomass produced was 33.4% less. When combined with different levels

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Table 4.2: Production of chlorophyll a (μg l^{-1}) by *S.elongatus* upon exposure to salinity and pesticide – chlorpyriphos.

Pesticide	Salinity			
	Nil	5x10^{-3}	10x10^{-3}	15x10^{-3}
Nil	146.40	99.2	72.6	48.20
	150.80	98.1	73.1	47.40
	143.20	98.8	68.8	54.10
15mgl^{-1}	111.40	62.60	29.10	27.40
	112.90	61.80	31.80	28.10
	113.80	67.00	27.60	27.60
30mgl^{-1}	74.10	57.10	31.30	25.90
	73.80	54.70	27.40	24.70
	69.30	58.00	28.60	29.80

Figure 4.2: Growth of *S.elongatus* measured as chlorophyll a (μg l^{-1}) upon exposure to salinity and pesticide – chlorpyriphos.
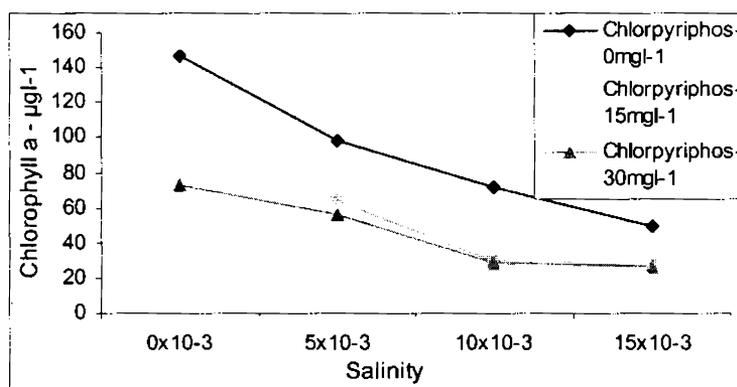

Table 4.3: ANOVA on data of growth of *S.elongatus* measured as chlorophyll a (μg l^{-1}) upon exposure to salinity and pesticide – chlorpyriphos.

Source of Variation	SS	Df	MS	F	P-value	F crit
Chlorpyriphos	13312	2	6656	1157.6	1.36117E-24	3.4028
Salinity	31735	3	10578	1839.7	1.74509E-28	3.0088
Interaction	2641.8	6	440.3	76.573	1.85664E-14	2.5082

of salinity, the yield of chlorophyll a got reduced as the salinity increased. Thus at 15 mg l^{-1} of pesticide and salinity 5×10^{-3}, the growth was inhibited by > 50% of the non saline medium (Table 4.2, Figure 4.2). The interaction between salinity and chlorpyriphos toxicity was highly significant (Table 4.3).

Monochrotophos

Monochrotophos applied to medium at a concentration of 45mg l^{-1} effected 26.0% inhibition on the growth of *S.elongatus* in BG 11 medium. The effect was found to be 51.4% inhibition when the pesticide was added at a concentration of 90 mg l^{-1}. Salinity of the medium reduced the growth of the algae in the presence as well as absence of pesticide (Table 4.4).

Table 4.4: Production of chlorophyll a ($\mu g\ l^{-1}$) by *S.elongatus* upon exposure to salinity and pesticide – monochrotophos.

Pesticide	Salinity			
	Nil	5×10^{-3}	10×10^{-3}	15×10^{-3}
Nil	125.90	84.25	67.75	42.90
	123.80	85.38	63.16	43.80
	128.00	83.12	72.39	42.10
45mgl^{-1}	93.70	74.80	57.80	41.40
	94.10	75.90	55.20	42.70
	91.80	77.30	56.80	38.30
90mgl^{-1}	62.70	44.30	35.80	29.70
	63.10	45.10	36.70	28.10
	57.80	48.30	35.80	29.80

The highest inhibition of growth was effected at 15×10^{-3} salinity and 90 mg l^{-1} pesticide, which was 76.9 %(Table 4.4; Figure 4.3). The individual effect of monochrotophos and the interaction with salinity was found to be significant (Table 4.5).

Figure 4.3: Growth of *S. elongatus* measured as chlorophyll *a* ($\mu g\ l^{-1}$) upon exposure to salinity and pesticide – monochrotophos.
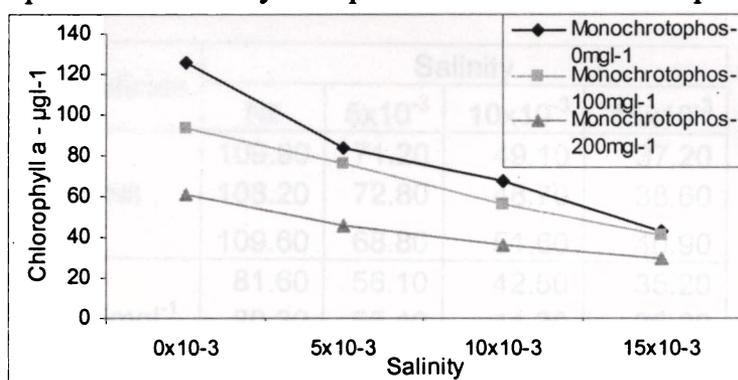

Table 4.5: ANOVA on data of growth of *S. elongatus* measured as chlorophyll *a* ($\mu g\ l^{-1}$) upon exposure to salinity and pesticide – monochrotophos.

Source of Variation	SS	df	MS	F	P-value	F crit
Monochrotophos	8463.5	2	4231.8	976.77	1.02097E-23	3.4028
Salinity	15226	3	5075.5	1171.5	3.80644E-26	3.0088
Interaction	2136.1	6	356.01	82.175	8.33967E-15	2.5082

Fenvalarate

Fenvalarate produced 26.2% growth inhibition at a concentration of 2.5 mg l^{-1}. The same concentration of the pesticide produced 49.9% of inhibition in combination with 5 $\times 10^{-3}$ salinity (Table 4.6). 5 mg l^{-1} of fenvalarate produced 53.1% inhibition, and showed similar trend of increasing inhibition when combined with increasing salinity (Figure 4.4). The interaction of the two factors was found to be significant (Table 4.7).

Table 4.6: Production of chlorophyll a (μg l^{-1}) by *S.elongatus* upon exposure to salinity and pesticide – fenvalarate.

Pesticide	Salinity			
	Nil	5x10^{-3}	10x10^{-3}	15x10^{-3}
Nil	109.90	71.20	49.10	37.20
	108.20	72.80	48.70	38.60
	109.60	68.80	51.60	40.90
2.5mgl^{-1}	81.60	56.10	42.50	35.20
	80.30	55.40	41.20	36.20
	79.60	52.60	43.00	29.80
5mgl^{-1}	50.80	42.40	33.10	27.20
	51.70	40.80	34.70	29.40
	51.10	41.00	34.80	33.40

Figure 4.4: Growth of *S.elongatus* measured as chlorophyll a (μgl^{-1}) upon exposure to salinity and pesticide – fenvalarate.
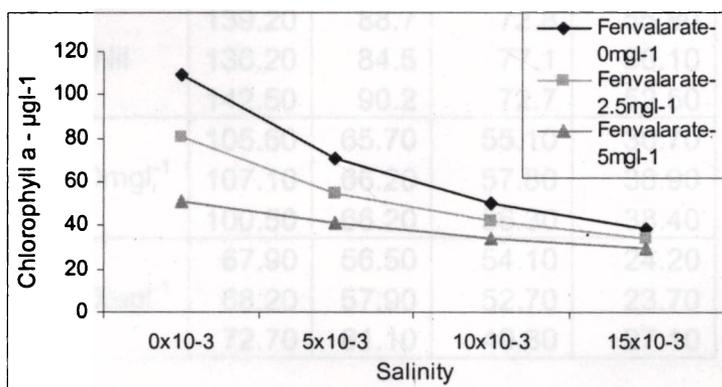
Table 4.7: ANOVA on data of growth of *S.elongatus* measured as chlorophyll a (μgl^{-1}) upon exposure to salinity and pesticide – fenvalarate.

Source of Variation	SS	df	MS	F	P-value	F crit
Fenvalarate	4711	2	2355.5	710.68	4E-22	3.4028
Salinity	11028	3	3676.1	1109.1	7E-26	3.0088
Interaction	2138.6	6	356.43	107.54	4E-16	2.5082

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Malathion

Malathion caused 25% and 50.4% growth inhibition when added to *S.elongatus* at a concentration of 100mgl^{-1} and 200mgl^{-1} respectively (Table 4.8). The growth inhibition increased upon increasing levels of salinity. The trend was the same in both pesticide levels (Figure 4.5). The growth inhibition caused by pesticide and salinity were found to be significant (Table 4.9) and their combined effect also was found to be having F value 94.798 against a critical value of 2.508.

Table 4.8: Production of chlorophyll *a* (μg l^{-1}) by *S.elongatus* upon exposure to salinity and pesticide – malathion.

| Pesticide	Salinity			
	Nil	5x10^{-3}	10x10^{-3}	15x10^{-3}
Nil	139.20	88.7	72.8	55.80
	136.20	84.5	77.1	56.10
	142.50	90.2	72.7	52.50
100mgl^{-1}	105.60	65.70	55.10	36.70
	107.10	66.20	57.80	38.90
	100.50	66.20	56.30	38.40
200mgl^{-1}	67.90	56.50	54.10	24.20
	68.20	57.90	52.70	23.70
	72.70	61.10	49.80	27.10

Table 4.9: ANOVA on data of growth of *S.elongatus* measured as chlorophyll *a* (μg l^{-1}) upon exposure to salinity and pesticide – malathion.

Source of Variation	SS	df	MS	F	P-value	F crit
Malathion	7746.2	2	3873.1	713.46	4.19565E-22	3.4028
Salinity	19870	3	6623.2	1220.1	2.3464E-26	3.0088
Interaction	3087.7	6	514.62	94.798	1.6358E-15	2.5082

Figure 4.5: Growth of *S.elongatus* measured as chlorophyll *a* (μgl^{-1}) upon exposure to salinity and pesticide – malathion.
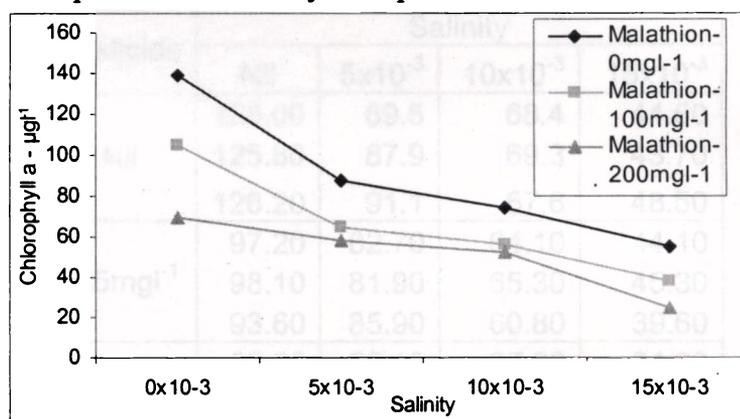
2,4-D

225 mg l^{-1} of 2,4-D was found to effect 23.5% growth inhibition on *S.elongatus* which was 5.8% less than the effect of 5×10^{-3} salinity. However on combination of these 2 stresses the inhibition was raised to 33.8% (Table 4.11). Similarly 450 mg l^{-1} of 2,4-D produced 50.9% inhibition which was 12.9% less than that of 15×10^{-3} salinity, and their combination produced 73.2% inhibition. All other combination produced similar trend (Figure 4.6). The ANOVA gave interaction F value of 78.741 against a critical value 2.508 (Table 4.10), indicating significant interaction.

Table 4.10: ANOVA on data of growth of *S.elongatus* measured as chlorophyll *a* (μgl^{-1}) upon exposure to salinity and pesticide – 2,4-D.

| Source of Variation	SS	df	MS	F	P-value	F crit
2,4-D	7062.3	2	3531.2	770.25	1.69838E-22	3.4028
Salinity	15154	3	5051.2	1101.8	7.9039E-26	3.0088
Interaction	2165.9	6	360.99	78.741	1.35343E-14	2.5082

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Table 4.11: Production of chlorophyll a (μg l^{-1}) by *S.elongatus* upon exposure to salinity and pesticide – 2,4-D.

Pesticide	Salinity			
	Nil	5×10^{-3}	10×10^{-3}	15×10^{-3}
Nil	126.00	89.5	68.4	44.60
	125.80	87.9	69.3	43.70
	126.20	91.1	67.6	48.50
225mgl⁻¹	97.20	82.70	64.10	44.10
	98.10	81.90	65.30	45.30
	93.60	85.90	60.80	39.60
450mgl⁻¹	62.80	58.10	37.20	34.20
	63.10	57.40	37.80	35.60
	62.50	63.30	41.70	31.60

Figure 4.6: Growth of *S.elongatus* measured as chlorophyll a (μg l^{-1}) upon exposure to salinity and pesticide – 2,4-D.
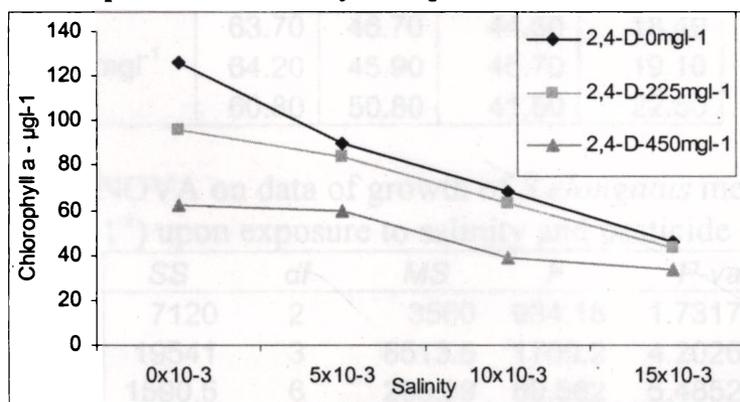
Glyphosate

Glyphosate was found to produce 25.9% and 50.6% growth inhibition by 2.5 mg l^{-1} and 5 mg l^{-1} concentrations respectively (Table 4.12). At salinity level 5×10^{-3}, the yield of chlorophyll a was $\sim$ 60% of the control, whereas in the presence of glyphosate (2.5mg l^{-1}), the chlorophyll a value was $\sim$ 47.5% of the control, clearly indicating that salinity and pesticide together has synergistic effect on the organism. Similar response was

observed at higher levels of pesticides and salinity (Table 4.12 and Figure 4.7). The significant interaction between glyphosate and salinity was provided by statistical analysis (Table 4.13).

Table 4.12: Production of chlorophyll a (μg l^{-1}) by *S.elongatus* upon exposure to salinity and pesticide – glyphosate.

Pesticide	Salinity			
	Nil	5x10^{-3}	10x10^{-3}	15x10^{-3}
Nil	125.40	78.1	71.1	40.20
	126.70	77.6	73.7	39.80
	121.40	73.2	70.9	38.50
2.5mgl^{-1}	95.80	58.70	58.40	26.20
	93.10	59.10	57.10	25.80
	94.60	61.90	57.30	23.30
5mgl^{-1}	63.70	46.70	44.50	18.40
	64.20	45.90	45.70	19.10
	60.80	50.80	41.50	22.50

Table 4.13: ANOVA on data of growth of *S.elongatus* measured as chlorophyll a (μg l^{-1}) upon exposure to salinity and pesticide – glyphosate

Source of Variation	SS	df	MS	F	P-value	F crit
Glyphosate	7120	2	3560	934.18	1.73178E-23	3.4028
Salinity	19541	3	6513.5	1709.2	4.20261E-28	3.0088
Interaction	1590.5	6	265.09	69.562	5.48526E-14	2.5082

Figure 4.7: Growth of *S.elongatus* measured as chlorophyll a (μg l^{-1}) upon exposure to salinity and pesticide – glyphosate:
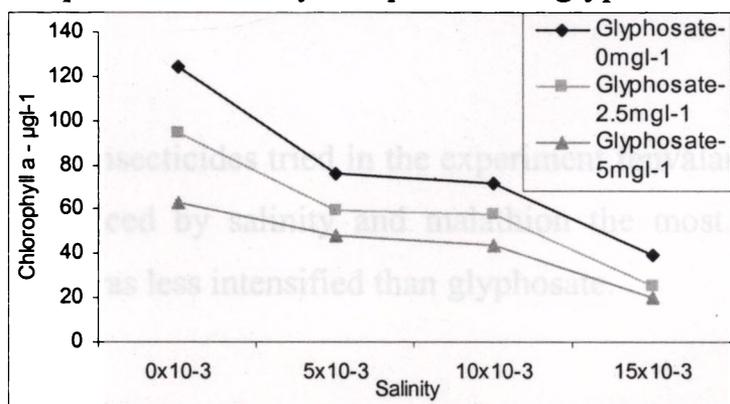
4.4 Discussion

As a group cyanobacteria exhibit considerable salinity tolerance and many are adapted to hyper-saline environment. Salinity affects organisms both directly through inhibitory effects on physiology and indirectly by affecting other environmental factors (Moisander *et al.* 2002).

In the experimental cultures, amended with different levels of salinity the pH was found to be increasing from 7.6 to up to 9.0. Cultures with lower salinity as well as higher salinity have moved more nearer to pH 9, though culture with maximum salinity (15×10^{-3}) remained around ph 8.9 itself. This might indicate a higher rate of photosynthesis in less saline cultures.

Pesticide and salinity had shown very significant levels of interference throughout the experiment. However the degree of toxicity of the different pesticides was not strictly linear to increasing salinity. So also the measure of interaction between the two factors, given as interaction F, varies from 69.5 to 107.5. Whereas salinity itself was inhibitory to the growth of the species, so also was pesticide with increasing dosage. The interaction was that at lower pesticide levels there is strong synergistic action on growth, while at higher levels of pesticide individual effects itself may sufficiently inhibit growth.

Of the four insecticides tried in the experiment fenvalarate was found to be least influenced by salinity and malathion the most. Of the two herbicides, 2,4-D was less intensified than glyphosate.

The general impression of the whole experiment was that salinity can modify the toxicity of pesticides in general. Insecticides and herbicides were, more or less, equally intensified. Staton *et al.* (2002) also have reported that there was significant interaction between salinity and pesticide in their study using Meiobenthic copepod (poppe). The pokkali fields and kuttanad fields, where paddy cultivation is done under the threat of encroachment of salinity, might turn out to be a high risk area for the microalgal population, as the agro-biocides might become aggressively toxic under saline environments.

Chapter 5

Summary and Conclusion

Microalgae are reckoned as important constituent of the paddy field agroecosystem. The soil microflora act as a go-between the soil and crop, make available more nutrients and increase the hospitality of soil to the crops. In spite of all, the existence of algae in paddy fields is not all that easy these days; for, sustainable paddy cultivation also means a sustainable pest management, and sustainable pest control depends heavily upon the use of pesticide products. Microalgae easily become the first, in the list of non-target organisms that are affected by pesticides. An ecotoxicological approach on a member of paddy field microflora was chosen to be the thrust area which needs systematic study. The sites selected for the purpose were (1) the low-lying saline fields were at Vytila, Ernakulam district (site-1) and (2) the upland fields at Thrikkakara, Ernakulam district (site-2).

The population size of microalgae in the paddy field, during the cropping seasons, was found to follow no specific pattern. There were random ebbs and highs in the number of algae, which varied from 520 g^{-1} to infinite. Therefore the generalization that cyanobacterial population gives a linear supply of nitrogenous matter to the soil is disputed. Application of

cyanobacteria as biofertilizer may not bring about the desirable changes in rain fed paddy field especially during the monsoon. The survey of pokkali field and upland field showed a good diversity within cyanobacterial population in the paddy field; 31 species belonging to 20 genera were encountered. The study has reaffirmed the ideal nature of paddy field for cyanobacterial growth.

A unicellular cyanobacterium was successfully isolated from pokkali fields. The isolate was characterized and identified as *Synechococcus elongatus*. It measured 5-6 * 2-2.5 μ and appeared as paired cylindrical cells. SEM studies have shown a smooth surface. TEM studies showed thylakoids, polyhedral bodies, phycobilisomes and gas filled vesicles. The absorption spectrum of chlorophyll has shown typical absorption maxima of chlorophyll *a* at 665 nm and 430 nm, and absence of chlorophyll *b* and chlorophyll *c*. Similar study on water soluble pigments have shown the characteristic peaks of C-phycocyanin (620 nm) and C-phycoerythrin (560 nm). The unicellular soil alga was very slow growing *in vitro* conditions, the maximum divisions per day was 0.14 and attained stationary phase around 36th day. The strain was found to tolerate pH ranging from 6 to 10. It did not show any antibiotic properties against *E.coli* and was sharply sensitive to ampicillin. The GC analysis of nitrogenase has shown no initiation of the enzyme activity in aerobic or anaerobic, dark or illuminated conditions.

The paddy field cyanobacterium *Synechococcus elongatus* was subjected to an elaborate pesticide toxicity study. The growth response to 10 different pesticides, commonly used by farmers was estimated. Chlorophyll

a, cell count and primary productivity were the parameters of measurement. It was found that a synthetic pyrethroid insecticide – fenvalarate was very toxic (EC_{50} – 5 mg l^{-1}) to *S.elongatus*. Organophosphates were generally better tolerated, malathion had EC_{50} – 200 mg l^{-1}, monochrotophos and chlorpyriphos had EC_{50} – 90 mg l^{-1} and EC_{50} – 30 mg l^{-1} respectively. Herbicide 2,4-D (EC_{50} – 450 mg l^{-1}) was well tolerated and even acted as biostimulant at lower concentrations. Whereas glyphosate, another herbicide, was rather highly toxic (EC_{50} – 5 mg l^{-1}) to the isolate. Fungicides used were chemicals belonging to the category carbamates. Generally their toxicity was very high and recorded an EC_{50} – captan 70 mg l^{-1}, carbendazim 5 mg l^{-1} and mancozeb 25 mg l^{-1}. The only bactericide tried was bacterimycin. The drug is not very popular in paddy cultivation, yet, since it is designed to kill prokaryotic organisms, was tried in the experiments. Bacterimycin had very severe toxic effect on *S.elongatus* and the EC_{50} was >1 mg l^{-1}. The entire tests were done at 2 stages – a range finding test and a definitive test. The growth response of *S.elongatus* was compared to that of *Chlorella pyrenoidosa*. Most of the pesticides were found to be more toxic to chlorophyceae member than cyanophyceae member, except 2,4-D.

Algalization of agricultural fields has reached a state of art technology. The manifold effect of algalization are addition of nitrogenous matter to soil, aggregation of soil particles and hence improvement in soil aeration and water movement, conversion of non available phosphorus into available phosphate, general increase of soil humic matter, reduction in the oxidizable matter like sulfate and iron *etc.* Screening of algal isolate to detect the most effective strain and bulk production of the effective strain are on the cards. The microcosm experiment on soil algalization has shown an

increase in soil biomass. Also an increase in soil pH and reduction in soil EC were observed. Algalization with unicellular *Synechococcus elongatus* can improve the soil conditions generally though not in nitrogenous matter.

Accumulation of persistent pesticide residues in the soil is the most awesome aftermath of regular and excessive use of pesticides for crop protection. Though the accumulated residues are not at all protective to the crops, they cause a lot of environmental hazards by harming the non target organisms in the agroecosystem, even during the non cropping seasons. Therefore an abatement of the pesticide residues of the soil is the need of the hour. Biodegradation of pesticide residues by the metabolic activities of microalgae is one possible way of containing the damage. Algalization experiment at the microcosm level was carried out to see if the soil fortified with known quantities of insecticides get degraded or not. The change in level of pesticide residue in algalized soil and non-algalized soil were measured with GC. However the present isolate did not have a role in degradation of chlorpyriphos and monochrotophos residues in soil, within the period of 15 days growth in soil microcosm.

It is well established that the accumulated pesticide molecules in soil do not remain in a stationary phase. They get transported in water soluble forms, generally called leaching. The leachates reach all those nearby water bodies and eventually reach the ocean. The leachates even reach the ground water as well and there are reports of very high levels of pesticide residues in the water from deep tube wells in the neighborhood of paddy fields. Soil at a microcosm level was fortified with different pesticides and leachates were extracted from them. The toxicity of these leachates was bioassayed

by 96 h definitive test using *Synechococcus elongatus*. Significant levels of toxicity were obtained in the leachates of fenvalarate, malathion and 2,4-D. Algalization reduced the leachate toxicity of malathion and 2,4-D, indicating a metabolic degradation of these pesticide molecules. Whereas toxicity of fenvalarate was increased, indicating that degradation products of the pyrethroid molecules have amplified toxicity during the immediate days of algal growth. Glyphosate either did not leach out or the leachate was harmless, as it supported the growth of the test species to the same extent as in standard growth medium.

Pesticides are not applied directly at the pokkali fields; however these saline, low lands could be the potential sinks of most of the pollutants that come through water run off from the uplands. Therefore pesticides used in the upland paddy fields might come to act in the delicate ecosystem of pokkali fields as well. However, the toxic effects of pesticide molecules on microalgae already under stress of salinity might be in a state of modification. Therefore a study on this regard – interaction of pesticide toxicity and salinity stress was designed during the present investigation. *Synechococcus elongatus* was cultured in media amended to different levels of salinity and different levels of pesticides. It was found that the toxicity of all the insecticides and herbicides were magnified under saline condition. Therefore, it is reasoned that microalgae of pokkali fields and Kuttanad fields are at greater risks of pesticide hazards under salinity prone conditions.

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