ISOLATION AND CHARACTERIZATION OF HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES FOR USE AS A BIOFERTILIZER FOR RICE

Thesis submitted in part fulfilment of the requirements for the degree of Master of Science (Agriculture) in Agricultural Microbiology to the Tamil Nadu Agricultural University, Coimbatore - 641 003

By

G. SELVAKUMAR, B.Sc. (Ag.)

(I.D.NO. 97-605-007)

CENTRE FOR ADVANCED STUDIES IN AGRICULTURAL MICROBIOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE - 641 003.

1999

CERTIFICATE

This is to certify that the thesis entitled **"ISOLATION AND CHARACTERIZATION OF HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES FOR USE AS A BIOFERTILIZER FOR RICE "** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in AGRICULTURAL MICROBIOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a bonafide record of research work carried out by **Mr. G. SELVAKUMAR** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Date : 20/5/99
Place : Coimbatore

(Dr. G. GOPALASWAMY)
Chairman

APPROVED BY

Chairman : **(Dr. G. GOPALASWAMY)**

Members :

(Prof. Dr. S. KANNAIYAN)

(Dr. KAPIL DEO N. SINGH)

Date : 13-7-99

(External Examiner)

ABSTRACT

ISOLATION AND CHARACTERIZATION OF HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES FOR USE AS A BIOFERTILIZER FOR RICE

By

G. SELVAKUMAR

Degree : Master of Science (Agriculture) in Agricultural Microbiology

Chairman : **Dr. G. Gopalaswamy**
Associate Professor
(Agricultural Microbiology)
Centre for Plant Molecular Biology
Tamil Nadu Agricultural University
Coimbatore - 641 003.

1999

Thirteen cyanobacterial isolates belonging to the genera *Anabaena, Nostoc, Oscillatoria* and *Westiellopsis* were isolated from soils amended with herbicides and purified. Six cyanobacterial isolates *viz.*, *Anabaena*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria*-HT-SGK-1, *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 were selected for further study. Their growth performance and biochemical constituents *viz.*, chlorophyll content, ammonia excretion, phycobiliproteins, and protein content were assessed at 21 days after inoculation. Among the cyanobacterial isolates *Westiellopsis*-HT-SGK-2 registered maximum growth and biochemical constituents.

The effect of the most widely used rice field herbicide butachlor in influencing the growth and biochemical constituents of the cyanobacterial isolates

was tested by growing them in BG-11 medium supplemented with butachlor at various concentrations *viz.*, 0, 3, 6, 9 and 12 ppm. *Westiellopsis* -HT-SGK-1 and *Westiellopsis* - HT-SGK-2 were found to tolerate the butachlor concentrations even upto 12 ppm by registering higher growth and biochemical constituents. The cyanobacterial isolate *Anabaena*-HT-SGK-2 could tolerate upto 9 ppm of butachlor.

Molecular characterization of the herbicide tolerant cyanobacterial isolates revealed the absence of plasmids in any of the isolates. However the chromosomal DNA appeared as a single intact band.

The effect of inoculation of the herbicide tolerant cyanobacterial isolates on the growth of ADT 36 rice seedlings was studied. Inoculation of composite culture of the herbicide tolerant cyanobacterial isolates significantly increased the plant height, chlorophyll content and total nitrogen of the rice seedlings over individual inoculation both in the presence and absence of butachlor.

The performance of composite inoculum was tested under graded levels of N fertilizer. Inoculation of composite culture along with 75% N application as urea significantly increased the plant growth, tiller production, total N, N uptake, grain and straw yield of ADT 36 rice on par with 100% N application alone. The results of the present study clearly demonstrated the superiority of the herbicide tolerant cyanobacterial isolates in augmenting rice yield in the presence of herbicide.

ACKNOWLEDGEMENT

I wish to thank my Chairman Dr. G. Gopalaswamy, Associate Professor (Agricultural Microbiology) for entrusting this research work to me. His benevolence, encouragement, valuable suggestions and constructive criticisms were important ingredients for the successful completion of this study, I'm sure that the values he has instilled in me will hold good for the rest of my career.

With reverence, I place on record my sincere gratitude to Prof. Dr.S.Kannaiyan, Dean (Agriculture) for providing the best possible facilities for my research. His wise counsel and dextrous comments as a member of the advisory committee were of immense value.

I am indebted to Dr.Kapil Deo N. Singh, Assistant Professor, CPMB and member of the advisory committee for his valuable guidance.

My heartfelt gratitude is due to Dr. P.Santhanakrishnan, Professor and Head, Department of Agricultural Microbiology for his generous help and encouragement. I wish to thank Dr. S.Gunasekaran, PG Coordinator, Department of Agricultural Microbiology for extending his guidance and help during the period of my study. My sincere thanks to all faculty members of the Department of Agricultural Microbiology for their guidance and help.

I place on record my sincere gratitude to Dr. S.Sadasivam, Director, CPMB for enabling me to use the facilities available in CPMB.

Words are inadequate to express the gratitude I owe to Dr. K.Kumar, Associate Professor (Microbiology) for extending every possible help at any hour during the study period.

My heartfelt gratitude is due to Dr. J. Venkitapirabhu, Assistant Professor (Extension) and Mr. L.Arul, Assistant Professor (CPMB) for guiding me through this study period.

I would be failing in my duty If I don't acknowledge the unstinted help extended by Dr.J.A.J.Raja, Research Associate, CPMB, Mr.T.Parimelazhagan, Mr.N. Senthil, Mr.N.C. Sivakumar, and Mr.T. Thirumurugan, Senior Research Fellows of CPMB. I thank

Ms.P.Yasodha, Junior Research Fellow of Azolla Lab for helping me a lot. I recall with gratitude the guidance of Mr.Viren Konde, Ms.S. Sujatha Lilly and Mr.B. Tamilselvam, Ph.D.Scholars of Azolla lab.

The help and guidance extended by Prof. S. Shanmugasundaram, Professor and Head, Department of Microbial Technology, MKU, Madurai and Dr. Suguna Shanmugasundaram in carrying out a portion of the work is being gratefully acknowledged.

On a personal note I thank my dear friends K.A.Haridass, K.R.Prince, and A.Harish Chandran for standing by me even through the most testing times. The co-operation extended by R. Karthikeyan, K. Sivakumar, K. Sivasubramaniam, D. Bala, T.S. Anbu, Lovely, K.Jeyaram, R.Parthasarathi, N. Vigneswaran, B. Benvit, K.M.Sellamuthu, P.Murali, M.Senthil Kumar, R. Sugumar and V.Rajkumar was instrumental at one or other phase in this work seeing the dawn of the day. The help extended by K.B. Rangaraj was greatly instrumental in the successful completion of the thesis.

In this joyous moment, I'm happy to recall the encouragement and support I received from Sasi and Subashini in times of need..

I owe my gratitude to the staff of Azolla lab for their kind help and cooperation.

A word of appreciation is due to M/s.Shree Nandha Systems, Coimbatore for the neat and excellent execution of the thesis.

The love, care, patience, perseverance, sacrifices and unstinted support of my parents Mr. C.Govindan and Dr.(Mrs.)Bhagavathi Govindan are what I am today. What little I do I dedicate it to them. The affection showered upon me by my loving sister G. Meenakumari has no parallel.

Above all the divine grace and blessings of My Lord atop the holy eighteen steps has carried me through many testing periods. I bow in reverence at the lotus feet of My Lord for enabling me to complete this piece of work successfully.

G. Selvakumar

(G.SELVAKUMAR)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

Rice is the staple food crop of most Asian nations and contributes upto 20 per cent of the world food grain production (Stangel, 1984). In India, it is cultivated in an area of 48.38 m.ha with an annual production of 81.31 m.t and a productivity level of 1879 kg ha^{-1} (Paroda, 1999). As our population increases at the rate of 2.2 per cent per annum, the current production and productivity levels need to be doubled to feed the human population in the next millennium.

Nitrogen is the most important determining factor in successful rice cultivation. The nitrogen supply for rice crop is largely met through the expensive inorganic chemical fertilizers which are not ecofriendly. Despite such large investments, the utilization efficiency of these fertilizers by the rice crop is only around 40%. Developing nations can ill afford to spend such exhorbitant costs to meet their fertilizer bills.

It is in this context ,biofertilizers are considered a more viable, realistic, ecofriendly and inexpensive alternative to supply nitrogen to rice crop on a sustainable basis. *Azolla*, cyanobacteria and other heterotropic bacteria are known to fix the atmospheric nitrogen in the rice field ecosystem. Among them, the cyanobacteria commonly referred as blue green algae have colonized the rice fields for centuries and are known to fix nitrogen both under free living as well as in symbiotic conditions (Singh, 1961). The fertility of the tropical rice fields has been sustained for centuries due to the activities of these diazotrophs.

Cyanobacteria in the rice fields are subjected to variety of environmental and applied stresses. And often these stress factors inhibit the cyanobacteria from realizing their full potential. Present day agriculture involves the use of several chemicals and herbicides which form an integral part of the package of the rice

cultivation in the the low lands due to labour savings and high efficiency in controlling several pugnacious weeds. And despite increasing efforts towards obviating the use of these chemicals, we are still dependent on the herbicides which are ultimately deposited in the soil (Padhy, 1985). Very often cyanobacteria which are photosynthetic organisms are at the receiving end of these herbicides which specifically alter the photosystems of the target and non target organisms alike.

Most studies on the effect of herbicides on cyanobacteria conducted over the past few decades mostly pertain to study the changes in soil due to herbicides(Mishra, 1989). Recent studies have been aimed to study the changes in biochemical and physiological parameters such as nitrogen fixation(Kapoor and Sharma,1980;Kaushik and Venkataraman,1983).

The commonly used rice field herbicides are butachlor,anilophos, thiobencarb, propanil and 2,4-D. Among them butachlor is the most popular and widely used in the paddy growing areas.. Singh and Vaishampayan (1978) observed variation in the response to butachlor at the generic level of cyanobacteria. According to them *Anabaena dolionum* is inhibited in protein synthesis, phycobiliproteins and nitrogen fixation by 5 ppm of butachlor, while there was no marked inhibition of *Nostoc muscorum* even at 20 ppm level of butachlor, under *in vitro* conditions without any significant impairment of cell activity. Zagar and Dar (1990) reported that under field conditions most cyanobacterial genera could tolerate upto 70 ppm of butachlor.

With a view to improve the efficiency, and performance, isolation of cyanobacteria from the truly representative ecosystem has gained momentum over the years . Site specific strains are known to perform better than introduced strains.Amsaveni(1995) isolated cyanobacterial strains from the saline soils of TamilNadu and recorded their superior performance. Tamilselvam (1998) isolated cyanobacterial strains from the acid soils of Tamil Nadu and observed their superior

performance both under *in vitro* and *in vivo* conditions. Much of the study conducted so far was by testing the efficacy of some standard cyanobacterial cultures to find out their herbicide tolerance. If cyanobacterial strains could be isolated from a particular stress environment they will bring forth the desired response in the rice ecosystem. With this aim the present study was taken up with the following objectives.

i) To standardize the protocol for isolation and purification of cyanobacteria from herbicide amended paddy soils.

ii) To study the effect of the herbicide butachlor on the growth, nitrogen fixation and ammonia excretion by herbicide tolerant cyanobacteria isolates.

iii) To study the effect of butachlor on the pigments and total protein content of the isolates.

iv) To carry out molecular characterization of the herbicide tolerant cyanobacterial isolates and to screen for the presence of plasmids.

v) To study the effect of inoculation of the herbicide tolerant isolates on rice seedlings.

vi) To study the effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N fertilizer on the yield of rice crop.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 CYANOBACTERIA-GENERAL

Biological Nitrogen Fixation (BNF) is considered as a vital process in the rice ecosystem by which the nitrogen balance is maintained. Diazotrophy in the wetlands is mainly due to the activities of *Azolla*, cyanobacteria and other heterotrophic bacteria. The cyanobacteria which are commonly referred to as blue green algae have colonized earth for over 3 billion years. Primarily they are photosynthetic organisms which reduce CO_2 and evolve O_2. A great deal of similarity is observed between the photosynthetic features of cyanobacteria and higher plants. The cyanobacteria can as well be considered the evolutionary predecessors of modern day cellular organelles which is evident by the molecular similarity between the two (Haselkorn, 1978).

The unique feature of cyanobacteria is their occurrence in a wide range of habitats ranging from freshwater, acidic soils, alkaline soils, thermal springs, tundras, desert soils, fertile soils, sub aerial habitats like tree trunks and as symbionts with bryophytes, pteridophytes and higher plants (Brock, 1973).

Cyanobacteria have been isolated from diverse sources like saline soils (Amsaveni, 1995), acid soils (Aiyer, 1965; Madhusoodhanan and Dominic, 1995; Tamilselvam, 1998), hot springs (Ward *et al.*, 1997)and coastal swamps (Komarek, 1998).

Earlier approaches in cyanobacterial taxonomy largely depended on the morphological features of the cyanobacteria (Desikachary, 1959). Margheri and Tomaselli (1995) proposed a system of classification based on the type of fatty acids present in the cyanobacteria. With recent advances in scientific research, it is possible to classify the cyanobacterial isolates by SDS-PAGE and 16s rDNA sequencing (Palinska *et al.*, 1996).

2.2 PHOTOSYNTHETIC PIGMENTS OF CYANOBACTERIA

The photosynthetic system of prokaryotic cyanobacteria bears a close resemblance to the eukaryotic organisms (Carr and Whitton, 1982; Pakrasi and Sherman, 1984; Abe *et al.*, 1988). The major photosynthetic pigments are the chlorophyll-a, phycocyanin, allophycocyanin, phycoerythrin, and other phycobiliproteins (Glazer, 1977; Amla 1979).

Chlorophyll-a is the major photosynthetic pigment in cyanobacteria. The chlorophylla-a pigment is present in the thylakoids of the cyanobacteria, (Pinevich, 1986). Rathore *et al.* (1993) found that the chlorophyll-a content was affected by the deficiency of molybdenum.

Phycocyanin and allophycocyanin are present universally while allophycocyanin B is apparently present in most cyanobacteria (Bogorad, 1975; Bryant *et al.*, 1976). Phycoerythrocyanin similar to phycocyanin in function and phycoerythrin in structure is limited in distribution among the cyanobacterial genera (Bryant *et al.*, 1976). More C-phycoerythrin is accumulated intercellularly in relation to chlorophyll when cyanobacteria were grown under standard conditions (Erokhina, 1990).

The phycobiliproteins are brilliantly coloured, highly fluorescent light harvesting compounds of cyanobacteria, algae and cryptomonads (Gantl, 1980; Glazer, 1977). In *Anabaena cylindrica* nearly 40 per cent of the proteins are composed of phycobili proteins. The phycobiliproteins constitute the energy transfer chain of the cell. Within the cell, the light energy trapped by the phycobiliproteins is transferred to chlorophyll-a with an efficiency approaching 100 per cent. Recently the existence of a specific phycobilisome photosystem I has been reported in *Synechocystis* by which light transfer from phycobilisomes to PS I can be direct rather than a spill over from PS II (Millineaux, 1994).

Carotenoids, a yellow colour pigment serves as a light harvesting pigment and is intimately associated with photosynthetic reaction centres (Siefermann - Harms, 1987). The carotenoids play a major role in protecting against the potentially lethal photooxidative damage (Koyama, 1991). They also serve as substrates for the biosynthesis of absissic acid (Parry and Horgan, 1991). Besides they also dissipate the excess light captured by the light harvesting antenna (Demming-Adams and Adams, 1992).

Rucker *et al*, (1995) studied the responses of carotenoids and chlorophyll to variations in growth limiting factors such as nitrogen, phosphorus and light in the filamentous cyanobacteria *viz.*, *Planicothrix agardis, Limnothrix redekli* and *Aphanizomenon gracile*. They observed that the growth conditions had no detectable influence on the carotenoid content. They also found that the adaptive changes due to nutrient limitation was independent of the chlorophyll-a content.

The phenyl carbamate herbicides caused a decrease in C-phycoerythrin pigments levels and chlorophyll content, simultaneously recording an increase in C-phycocyanin content at 3 ppm level (Wright, 1978). Inhibition of the PSI activity of *Anabaena* upto 45% was observed under the influence of butachlor at 100 ppm level (Vaishampayam, 1985). Gustavson and Wanberg (1995) observed a reduction in the photosynthetic activity of micro algal communities treated with atrazine.

The pH of the medium largely affects the pigment synthesis. At pH 5 chlorophyll synthesis was low in the initial stages after inoculation (Subramanian and Shanmugasundaram, 1987).

Anand *et al.* (1994) reported that the cyanobacteria *Nostoc pisunale* and *Tolypothrix tenuis* released phycobilin pigments in the extra cellular medium at 2.5-3.5 per cent salinities.

2.3. NITROGEN FIXATION BY CYANOBACTERIA

Diazotrophy by cyanobacteria was reported in 1849 soon after the discovery of legume root nodule bacteria (Waksman, 1962). Drews (1928) established nitrogen fixation by axenic cyanobacterial cultures.

The nitrogen fixing ability of the cyanobacteria is closely related to the presence of specialized cells called heterocysts which do not evolve oxygen. It is in these cells nitrogenase a highly oxygen sensitive enzyme is protected. The enzyme requires a source of ATP and reductant for its activity (Shi and Hall, 1988). Nitrogenase requires two electrons to reduce acetylene to ethylene and seven electrons to reduce dinitrogen to ammonia along with 16 moles of ATP. The optimal ATP concentration for cyanobacterial nitrogenase activity is from 2-3 mM of ATP.

All heterocystous types of cyanobacteria can fix atmospheric nitrogen aerobically but only a few non heterocystous filamentous and unicellular cyanobacteria posses this property (Gallon, 1980). A few sheathless unicellular cyanobacteria identified as *Synechococcus* sp were found to exhibit high growth rates and nitrogen fixing activities under aerobic conditions (Huang and Chow, 1986; Leon *et al.*, 1986).

A metabolic inter relationship appears to exist between the photoassimilation of organic substrates and nitrogen fixation in cyanobacteria (Fogg, 1949; Ernst *et al.*, 1984). Under nitrogen saturation and excess carbon the cyanobacteria are known to accumulate glycogen (Chao and Bower, 1971; Ernst *et al.*, 1984). This could serve as a carbon pool for the protein synthesis and reduction of acetylene to ethylene (Rippka *et al*, 1979; Ernst *et al.*, 1984).

By using the acetylene reduction assay, Roger and Kulasooriya (1980), estimated the nitrogen fixation by cyanobacteria from a few kg to 89 kg ha^{-1}.

A bloom of cyanobacteria is believed to contribute less than 10 kg ha^{-1} while a dense bloom contributes 10-20 kg N ha^{-1} under field conditions.

2.4. FACTORS AFFECTING CYANOBACTERIAL NITROGEN FIXATION

Cyanobacteria thrive in a wide range of temperature and thermophiles having tolerance upto 65°C have been recorded. Depending on the time of the growing season, the temperature optimum for nitrogenase activity was found to vary between 15°C to less than 20°C. The nitrogenase activity was found to be higher in the artic region than in the temperate region. In the tundra region, the nitrogen fixed by *Nostoc commune* which is the predominant cyanobacterial species has been estimated as 0.22 to 23.7 n mol of ethylene per hour (Liengen and Olsen, 1997).

The inhibitory effect of atmospheric oxygen on nitrogen fixation was noted during the early studies. The addition of a reducing agent like sulfhydryl increased the N uptake while subatmospheric levels of oxygen could increase the nitrogen fixation by *Anabaena flosaquae* and *Nostoc muscorum*. Conversely oxygen levels above the atmospheric levels caused a marked inhibition of the nitrogenase activity (Stewart and Pearson, 1960).

Direct relation is noted between the light intensity and nitrogenase activity (Tsygan Korl *et al.*, 1992). Markov *et al.*, (1992) reported that high light intensity could cause a decline in the rate of O_2 evolution accompanied by enhanced nitrogenase activity in *Anabaena variabilis* with irreversible effects. The *Calothrix* strain D 764 showed higher nitrogenase activity when exposed to any particular light flux followed by a dark period than with continuous illumination at the same flux (Islam and Whitton, 1992). Variation in light quality triggers cellular responses and differentiation in several cyanobacterial genera. A cyclic decrease in photosynthetic oxygen evolution under nitrogen fixing conditions was noticed when cyanobacteria were exposed to alternating light and dark cycles (Roger *et al.*, 1994).

The quality and nature of any extraneous inorganic nitrogen sources also influences the cyanobacterial growth and nitrogen fixation.. Ammonium ions and to a lesser extent nitrate ions could suppress the development of heterocysts (Fogg, 1949; Prasperi *et al.*, 1992). Ammonium chloride at 1mM level could cause reduction in nitrogenase activity upto 80% within 24 hours. But reversion was possible in 24 hours on removal of the nitrogen source (Kaushik, 1987). Under field conditions, products of urea hydrolysis could cause supression of the nitrogenase activity (Singh *et al.*, 1995).

Activation of nitrogenase and diazotrophic growth requires molybdenum in cyanobacteria (Apte and Thomas, 1984). Synthesis of nitrogenase in *Anabaena cylindrica* does not require molybdenum (Hallenback and Benemann, 1980). The role of sodium in cyanobacterial nitrogenase is yet to be fully ascertained (Apte and Thomas, 1984).

Application of alachlor at 5-40 μg ml^{-1} affected the nitrogen fixation in *Nostoc* and *Anabaena* (Singh, 1972). On the other hand, 2,4-D application stimulated the growth and nitrogenase activity in *Anabaena* (Subramanian and Shanmugasundaram, 1987).

The nitrogenase activity was inhibited when the pH shifted from neutral to alkaline conditions. When *Anabaena variabilis* was transferred to a pH of 10, ammonium could inactivate nitrogenase due to a reversible modification of dinitrogen reductase (Reigh *et al.*, 1987; Reigh and Boger, 1989). Growth and nitrogen fixation of *Anabaena variabilis* were completely arrested as the pH progressed from pH 7 to 10 (Fonkes *et al.*, 1987).

2.5 AMMONIA EXCRETION BY CYANOBACTERIA

Ammonia is an inorganic form of nitrogen that is directly incorporated into the organic linkage and thus an obligate inter mediate in the utilization of other

inorganic nitrogen sources. Ammonia has its own inherent ability to leak out from the cell into the environment and detection of extracellular ammonia in the presence of nitrate or dinitrogen in cultures is rarely possible (Boussiba and Gibson, 1991).

The effective production of ammonium from dinitrogen by nitrogen fixing cyanobacteria requires (i) Prevention of incorporation of ammonia through the GS-GOGAT pathway (Wolk *et al.*, 1976) (ii) Over coming the antagonistic effect of ammonia on dinitrogen fixation (Guerrero *et al.*, 1982). Both these requisites are simultaneously fulfilled by interfering with the operation of the main ammonia assimilation pathway by glutamate analogs like MSX (L-methionine DL sulfoxamine) or PT (Phosphinothrionim) (2 amino L- (methyl phosphinyl) -butanic acids) (Stewart and Rowell, 1975; Ramos *et al.*, 1981 and Lea *et al.*, 1984). Filaments of *Anabaena* ATCC 33047 with MSX or PT have shown to excrete ammonia at higher rates with relatively high efficiency in a short incubation period.

Ammonium excretion by the cyanobacteria into the environment is influenced by the net rate at which the internal pool of ammonia is converted into amino groups. The internal pools of ammonia are of the order of 1mM and appear to be relatively constant (Rai *et al.*, 1984).

Newton and Tyler (1987) reported that herbicides that bind specifically to PS II greatly increased ammonia excretion by heterocystous cyanobacteria when inoculated with MSX. Tiwari *et al.*, (1991) have developed herbicide resistant mutant of *Gloeocapsa* that was capable of secreting ammonia into the environment.

Sugunarani (1997) showed that salt tolerant *Westiellopsis* sp. recorded the highest levels of ammonia excretion in the growing medium. Tamil selvam (1998) reported no inhibition in the ammonium excretion by the acid tolerant *Westiellopsis* even at a pH of 4.

2.6. CYANOBACTERIAL PLASMIDS

Cyanobacteria posses covalently closed circular (CCC) plasmids. Asato and Guinoza (1973) first reported the presence of plasmids in *Synechococcus* 6301. Subsequently plasmids were reported in *Agmenellum quadruplicatum* (Roberts and Koths, 1976) and a number of other unicellular cyanobacteria (Lau *et al.*, 1980)

The base composition of the plasmids DNA was significantly different from chromosomal DNA (Roberts and Koths, 1976). And the overall size of the cyanobacterial plasmids varied from 1.9 - 75 Md (Lau and Dolittle, 1979).

The role of plasmids in physiology is still unknown. Though several functions like heavy metal tolerance and the ability to produce toxins and gas vacoules have been associated with plasmids (Lau *et al.*, 1980). Mazur *et al.* (1980) clearly reported that plasmids of *Anabaena* 7120 do not carry identifiable nif genes. Nicolson *et al* (1995) reported two genes capable of cysteine biosynthesis in the pANL plasmid of *Synechococcus* sp. PCC 7942.

2.7. HERBICIDES ON CYANOBACTERIA

Modern day agriculture involves the successful integration of chemicals and biological agents which contribute to crop production. But often the applied chemicals have deleterious effects on the biological agents such as cyanobacteria. Adhikary (1998) reviewed the deleterious effects of pesticides on growth and nitrogen fixation by cyanobacteria and reasoned that it might be influenced by (i) cyanobacterial strains (ii) type and concentration of the pesticides used (iii) intra and inter specific strain variation.

The direct effects of herbicide toxicity concern changes in growth rate and specific metabolic rates of photosynthesis and respiration. Cyanobacteria which are photosynthetic organisms are the most affected since most herbicides interfere with the photosynthetic system of the cyanobacteria. Often very small concentrations are

required for the inhibition of a single species in culture and greater tolerance is known to occur in soil (Grossbard and Davies, 1976).

Certain groups of herbicides have toxicity which is selective for certain groups of microorganisms (Padhy, 1985). Herbicides may promote either directly or indirectly the growth of one or more types of microorganisms, the type favoured being either beneficial or undesirable in the paddy field ecosystem (Adhikary, 1998).

Rice production in the low land ecosystem involves the use of herbicides. The recommended herbicides for transplanted rice include the phenoxys (2,4-D, MCPA, etc), amides (butachlor, propanil), thiocarbamates (thiobencarb), dinitro anilines (trifluraline), pyrimidines (bentazon) and the heterocyclic organic compounds like oxadiacinon (Lales *et al.*, 1989). Recently a dithiophosphate group of herbicides (anilophos) has been recommended on a commercial scale. Among the herbicides, triazines, phenylureas, and bipyridyls are inhibitors of photosynthesis by affecting electron transport and morphology. While phenoxy compounds, phenyl carbamates and acyl anilides act as inhibitors not primarily affecting photosynthesis. Kitchen *et al.* (1981) reported that herbicide application inhibits chlorophyll synthesis prior to porpyrin ring formation thereby affecting many physiological processes. It is to be noted that herbicides that are inhibitors of photosynthesis were more active in soil. However not all herbicides inhibited the microbiological activity even at large doses (Venkateswaralu, 1993).

Gamble *et al.*, (1952) observed that *Tolypothrix tenuis* was inhibited by 0.5 ppm of 2,4-D under *in vitro* conditions. The cyanobacterium *Cylinderospermum licheniforme* could tolerate upto 400 ppm of 2,4-D on agar plate culture without significant reduction in growth (Avrick *et al.*, 1971). *Anabaena* and *Nostoc* could tolerate 1 and 5 ppm of 2,4-D respectively under *in vitro* conditions (Pillay and Tchan, 1972). Singh (1972) observed that *Cylinderospermum* could

tolerate a maximum of 800 μg ml^{-1} of 2,4-D under liquid culture condition while the tolerance level dropped down to 100 μg ml^{-1} in agar plates. Stimulation of growth of *Anbaena spiroides* and *Anabaena aphanizomenoides* by 10 ppm of 2, 4 D was noted under *in vitro* conditions. No adverse effect was seen on the growth and respiration of *Anabaena variabilis* by 10 μm of 2,4-D (Hauxby *et al.*, 1977).

Substituted urea compounds were found to be toxic to *Aulosira fertilissima* (Venkataraman and Rajyalakshmi, 1972). Cullimore and McCann (1977) observed that MCPA was most toxic to *Scytonema* sp even at low concentrations. Growth suppression of *Lyngbya biergi* and *Anabaena variabilis* was observed under *in vitro* conditions by 10 μM of fluometron but interestingly respiration was unaffected (Hauxby *et al.*, 1977). Mehta and Hauxby (1977) recorded cellular disintegration, and disruption of photosynthetic activities in most cyanobacterial genera due to the incorporation of 10 μM of fluometron. Singh *et al.* (1978) observed that the cyanobacterial cultures *Anabaena dolinum* and *Nostoc muscorum* were susceptible to 5-40 μg ml^{-1} of alachlor under *in vitro* conditions.

Swain *et al.*, (1994) observed that *Microcystis aeroginosa* could tolerate upto 500 μg ml^{-1} of the herbicide DCMU under *in vitro* conditions. Rather (1994) observed that butachlor and propanil at 0.01 and 0.05 mg ml^{-1} respectively gave an initial increase in the growth of *Anabaena* while higher concentrations were inhibitory. Most degradation products of atrazine were found to be more toxic than the original product it self in their action on *Anabaena inaequalis* (Glen, 1984).

The cyanobacterial response to field application of herbicides has been reported. Ishizawa and Matsuguchi, (1966) observed that the incorporation of pentachlorophenol in lime soils stimulated the growth of several nitrogen fixing cyanobacterial genera . Propanil did not exert any toxic effect on *Tolypothrix tenuis* and *Calothrix brevissima* when applied to the soil (Ibrahim, 1972). While *Aulosira*

fertilissima was stimulated due to the field application of several commonly used pesticides (Ahmed and Venkataraman, 1973). Venkataraman and Rajyalakshmi (1971) observed that *Tolypothrix tenuis* could tolerate upto 250 kg ha^{-1} of 2,4-D.

Gopalaswamy *et al.* (1994) reported that the herbicides anilophos and butachlor affected the growth of *Azolla* under field conditions. The reduction was relatively less under thiobencarb treatment.

2.8. CYANOBACTERIAL RESPONSE TO PESTICIDAL STRESS

Herbicide tolerance may be defined as a relative measure which may be low or high depending on the detoxifying metabolizing system involved. Butachlor belonging to the amide group of herbicides is often hydrolyzed by the amidase group of enzymes which are produced in the plant system. Similarly thiobencarb belonging to the thiocarbamate group of herbicides are also degraded by the amidases (Malcom Devine *et al.*, 1993). Several cyanobacteria are capable of metabolizing individual pesticides (Moore and Dorward, 1968). The cyanobacteria *Anacystis nidulans* was able to concentrate DDT 849 times from a growth medium containing 1 ppm of the pesticide (Gregory *et al.*, 1969). While *Anabaena cylindrica* and *Microcystis aeroginosa* were capable of accumulating DDT 238 times respectively from a growth medium containing 1 ppm of DDT (Vance and Drummond, 1969). Six species of cyanobacteria viz., *Microcoleus chthonoplaster* strain BA-I, *Anabaena* strains CA and IF, *Coccachloris elabans* strain 17a, *Nostoc* strain MAC, and *Aphanocapsa* strain 6714 were capable of metabolizing napthalene to 1 napthol under photoautotropic conditions (Cerniglia *et al.*, 1979).

The cyanobacterium *Cylinderosperum* could detoxify lethal levels of commercial formulations of carbaryl, carbofuran, ziram and mancozeb in one and two hour exposures. The cells collected from the flasks containing heavy dose of the pesticides after one and two hours could grow remarkably better than pesticide un-treated cells in fresh growth media (Panigrahy, 1984). The phenyl carbamate

compounds propham and chloropropham are hydrolysed to corresponding compounds by cyanobacteria (Wright, 1978).

2.9. CYANOBACTERIA AS A BIOFERTILIZER FOR RICE

The shortage of fertilizer nitrogen and escalating costs in recent years have led to the exploration of new avenues to augment rice productivity and maintain the fertility of the paddy soils on a long term basis. The agronomic potential by blue green algae was at first recognised by De (1939) who attributed the natural fertility of rice fields to nitrogen fixing blue green algae. The tropical rice fields have sustained their fertility largely due to cyanobacteria which fix atmospheric nitrogen under aerobic photosynthetic conditions (Singh, 1961; Roger and Kulasooriya, 1980). Reasonable estimates of the fixed nitrogen have been put at 25-30 kg ha^{-1} and their contribution to rice yield at 10-20% (Venkataraman, 1981).

The beneficial effect of algal inoculation on the grain yield of rice has been documented at a number of locations (Venkataraman, 1972; Jaganathan and Kannaiyan, 1977; Ahluwalia *et al.*, 1993), different agroclimatic conditions and soil types (Goyal and Venkataraman, 1971 and Kannaiyan *et al.*, 1982).

Kannaiyan (1978) developed technology for the mass scale cultivation of cyanobacteria and found that the soil based culture could survive for more than 15 months. When algal inoculation was done for more than three seasons the inoculated algae could establish well and the effect persisted for subsequent crops. The positive results of the field experiment have shown that the algal inoculation would supplement 25-30 kg N ha^{-1} per cropping season besides improving soil fertility (Kannaiyan, 1983).

An increase in the grain yield of upto 10-20% has been observed as a result of algal application (Venkataraman, 1981). Kannaiyan *et al.*, (1982) demonstrated that split application of top dressing nitrogen for rice did not affect the establishment

and nitrogen fixation by the inoculated cyanobacteria in rice fields. Combined inoculation of *Azolla* and cyanobacteria along with 72 kg N ha^{-1} recorded the maximum yield of grain (Yanni, 1992). Chirriv *et al.* (1995) reported that BGA at 20 kg on the 7th day after transplanting could save N fertilizer upto 50%.

Composite cultures of cyanobacteria were more effective than single cultures in improving the rice yield (Nayak *et al.*, 1996). Ghosh and Saha (1993) reported that algalization could improve the N uptake by rice grains. The effect of algalization were found to be maximum under tillering and grain formation stages.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. GENERAL

3.1.1. Location

All the laboratory and pot culture experiments were conducted respectively at *Azolla* Laboratory and Paddy Breeding Station of Tamil Nadu Agricultural University, Coimbatore, which is located at an altitude of 426.7 meters above mean sea level, 11°N latitude and 77°E longitude.

3.1.2. Agroclimatic conditions

The mean maximum and minimum temperatures prevailing at Coimbatore are 31.5°C and 21°C respectively. The mean relative humidity is 61.1 per cent and the annual rainfall is 674.2 mm.

3.1.3. Glasswares

The glasswares used were cleaned with chromic acid solution and finally washed with water. The glass wares thus cleaned were rinsed with distilled water before use.

3.1.4. Chemicals

The analytical reagent (AR) grade chemicals of BDH, E.Merck, Himedia, and Qualigens, were used for biochemical studies. Enzymes for molecular biological works were obtained from M/s. Bangalore Genei.

3.2. ISOLATION AND PURIFICATION OF CYANOBACTERIA FROM HERBICIDE AMENDED SOIL

3.2.1. Collection of soil samples

Soil samples were collected at random from the permanent herbicide amended plot of wetlands of Tamil Nadu Agricultural University, Coimbatore. They were dried, powdered and used for the isolation of cyanobacteria.

3.2.2. Isolation of cyanobacteria

One gram soil was added to 100 ml of 0.1 per cent single super phosphate solution previously sterilized in 250 ml Erlenmeyer flasks. The flasks were incubated in the poly net house with 3000 lux light intensity at 28 ± 1°C for 3-4 weeks.

3.2.3. Purification of cyanobacterial cultures

The cyanobacterial cultures were purified by repeated transfer to nitrogen free BG-11 medium atleast 3 to 4 times. Further purification was done with antibiotics as detailed below.

3.2.3.1. Purification with triple antibiotic solution

3.2.3.1.1. Preparation of triple antibiotic solution

One hundred mg of penicillin G (Na or K salt) and 50 mg of streptomycin were dissolved in 10 ml of distilled water. 10 mg of chloramphenicol was dissolved in 95 per cent ethanol and this solution was added to the penicillin G - streptomycin mixture.

3.2.3.1.2. Purification of cyanobacteria

One ml of freshly isolated cyanobacterial suspension was inoculated in 0.1 to 1 ml of triple antibiotic solution for 6 - 10 h to kill other bacteria and fungi. After incubation the cultures were centrifuged at 3000 rpm for 5 minutes and the supernatant containing the triple antibiotic solution was discarded. The pellets were resuspended in nitrogen free BG-11 medium aseptically.

3.2.3.2. Purification of cyanobacteria by agar plating

The algal cultures were purified either by streaking or by pour plating the homogenized suspension in nitrogen free solid BG-11 medium in sterile petri plates. The petriplates were incubated for a fortnight under 3000 lux light intensity at 28 ± 1°C.

3.2.3.3. Identification of unialgal cultures

Well developed unialgal cultures were examined under the binocular microscope (Euromex BM 1274) and identified using the cyanobacterial taxonomy hand books (Desikachary, 1959 and Anand, 1989).

3.2.3.4. Cyanobacterial cultures

Six herbicide tolerant cyanobacterial cultures viz., *Anabaena*-HT-SGK-1. *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria*-HT-SGK-1, W*estiellopsis*-HT-SGK-1, *Westiellopsis*-HT-SGK-2 isloated from herbicide amended paddy soils were used for the study.

3.3. GROWTH PERFORMANCE AND BIOCHEMICAL CONSTITUENTS OF CYANOBACTERIAL ISOLATES

The cyanobacterial isolates *viz.*, *Anabaena*-HT-SGK-1, *Anabaena* - HT-SGK-2, *Nostoc* - HT-SGK-1, *Oscillatoria* - HT-SGK-1, *Westiellopsis* - HT-SGK-1 and *Westiellopsis* - HT-SGK-2 were inoculated at 1 ml level in 100 ml sterile N-free BG-11 medium. The flasks were incubated under 3000 lux light intensity at 28 ± 1°C for a period of 21days. Observations were made on the 21 st day for growth, biomass production, ammonia excretion, chlorophyll-a, C-phycocyanin, C-phycoerythrin, allophycocyanin and protein content under *in vitro* conditions.

3.3.1. Growth performance of the cyanobacterial isolates

The growth performance of the cyanobacterial isolates in nitrogen free BG-11 medium was determined at by measuring the optical density at 750 nm in a Beckman DU-64 spectrophotometer against the uninoculated BG-11 medium as blank. The values were expressed as optical density at 750 nm.

3.3.2. Biomass production by the cyanobacterial isolates

One hundred ml of the cyanobacterial culture suspension was filtered through a preweighed Whatman No.1 filter paper previously dried at 60°C for

2 hours. The cyanobacterial culture along with the filter paper was dried in the oven at 60°C for 3 h till constant weight were recorded. The difference in weight was recorded and expressed as biomass of the cyanobacterial cultures. The results were expressed as μg of biomass produced per ml of the culture suspension.

3.3.3. Estimation of chlorophyll-a content in the cyanobacterial isolates

Chlorophyll-a content of the cyanobacterial isolates was determined by following the method of Talling and Driver (1961). Ten ml of the cyanobacterial suspension was taken and pelleted by centrifugation in a table top centrifuge at 5000 rpm for 5 minutes. The pellet was resuspended in 95 per cent ethanol and ground in a homogeneizer. The extract was allowed to settle in the dark at room temperature (28 ± 1°C) for a period of 30 minutes and centrifuged again for 5 minutes at 5000 rpm. The absorbance was then read at 665 nm in a Beckman DU-64 spectrophotometer against 95 per cent ethanol as blank. The chlorophyll content of the cyanobacterial cultures was calculated using the formula.

$$\text{Chlorophyll-a} = A_{665} / 8.6 \times \text{volume of ethanol.}$$

where A_{665} = Absorbance at 665 nm.

The chlorophyll-a content was expressed as μg mg^{-1} dry weight of the cyanobacterial isolates.

3.3.4. Ammonia Excretion by the cyanobacterial isolates

Ammonia excretion by the cyanobacterial isolates was estimated on the 21st day by following the method developed by Solarzono (1969).

Reagents

i) **Phenol reagent** : 10 g distilled phenol in 100 ml of 95 per cent ethanol.

ii) **Nitroprusside reagent** : 500 mg of sodium nitroprusside in 100 ml of distilled water.

iii) **Alkaline stock**: 20 g of trisodium citrate and 5g of sodium hydroxide in 100 ml of double distilled water.

iv) **Sodium Hypochlorite stock**: Sodium hypochlorite solution with 4 per cent available chlorine.

v) **Oxidizing Reagent**: Prepared freshly by mixing alkaline stock and sodium hypochlorite stock in 4 : 1 ratio.

Procedure

Three ml of the culture filtrate was taken in a clean test tube. To this 0.2 ml of phenol reagent, 0.2 ml of nitroprusside reagent and 0.5 ml freshly prepared oxidizing reagent were added. The tube was covered with aluminium foil and incubated for 1 hour at 28 ± 1°C in the dark with intermittent shaking. The intensity of the blue colour developed was read in at 620 nm in the Beckman DU-64 spectrophotometer against a reagent blank. The data were calibrated using a standard graph prepared at the time of estimation with pure ammonium chloride and the results were expressed as n moles of ammonia per ml of culture filtrate.

3.3.5. Estimation of phycobilin pigments in the cyanobacterial isolates

The phycobilin pigments *viz.*, C-phycocyanin, C-phycoerythrin and Allophycocyanin were estimated spectrophotometrically following the method of Bennet and Bogorad (1971). Ten ml of homogenized cyanobacterial culture grown under a light intensity of 3000 lux at 28 ± 1°C for 16/8 h day / night cycle were centrifuged at 5000 rpm for 5 minutes. The cyanobacterial pellet was washed and suspended in two to three ml of 0.05 M phosphate buffer (pH 6.8) and subjected to freezing and thawing. The pigment containing buffer was centrifuged at 5000 rpm for 5 minutes and the supernatant was stored for 3h. The pellet was again subjected to freezing and thawing till a colourless supernatant was obtained. The pigment absorbance was measured at 562, 615 and 652 nm in a Beckman DU-64 spectrophotometer against 0.05 M phosphate buffer as blank. The

concentration of C-phycocyanin, C-phycoerythrin, and Allophycocyanin were calculated using the following formula.

$$\text{C-phycocyanin (PC)} = \frac{E_{615} - 0.474\,(E_{652})}{5.34}$$

$$\text{Allophycocyanin (APC)} = \frac{E_{652} - 0.208\,(E_{615})}{5.09}$$

$$\text{C-phycoerythrin} = \frac{E_{562} - 2.41\,(PC) - 0.849(APC)}{9.62}$$

The phycobillin pigments present in the cyanobacterial isolotes were expressed as μg mg^{-1} on dry weight basis.

3.3.6. Estimation of protein content in the cyanobacterial isolates

Five ml of the cyanobacterial suspension was centrifuged at 5000 rpm for 10 minutes and the pellet homogenized with 0.1 M phosphate buffer (pH 7.5). The content was centrifuged and the supernatant collected. Protein content in the supernatant was estimated by following the method developed by Lowry *et al.* (1951).

Reagents

A = 2 per cent sodium carbonate in 0.1 N sodium hydroxide.

B = 0.5 per cent copper sulphate in 1 per cent sodium potassium tartarate.

C = Alkaline copper solution : 50 ml of A was mixed with 1.0 ml of B before use.

D = Folin ciocalteau reagent

E = Protein extract

The extracts (0.1 ml and 0.2 ml) were taken and the volume was made upto 1 ml with distilled water. A tube with distilled water served as blank. Five ml of

reagent C was added and mixed well followed by 0.5 ml of reagent D. The blue colour developed was read after 30 minutes in a Beckman DU-64 spectrophotometer at 660 nm. The standard graph was prepared with bovine serum albumin. The protein content was expressed as $\mu g\ mg^{-1}$ on dry weight basis.

3.4. HERBICIDE TOLERANCE STUDIES ON CYANOBACTERIA

3.4.1. Effect of butachlor on the growth and biochemical constituents of cyanobacterial isolates

The cyanobacterial isolates *viz.*, *Anabaena*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria* -HT-SGK-1, *Westiellopsis*-HT-SGK-1, and *Westiellopsis*-HT-SGK-2 were tested for their tolerance to butachlor by growing them in BG-11 medium supplemented with butachlor at 3.0, 6.0, 9.0 and 12.0 ppm concentrations. The flasks were incubated with 3000 lux light intensity at 28±1°C for 30 days period. Investigation for growth, biomass production, ammonia excretion, chlorophyll-a, C-phycocyanin, C-phycoerythrin, allophycocyanin, and protein content was made at 10 days intervals by following the procedures detailed earlier in section 3.3.

3.4.2. Assay of nitrogenase activity of the herbicide tolerant cyanobacterial isolates

The nitrogenase activity of the herbicide tolerant cyanobacterial isolates was estimated by the acetylene reduction assay (Hardy *et al.*, 1968).One hundred ml of cyanobacterial cultures grown in 0, 3, 6, 9 and 12 ppm of butachlor respectively were taken and blot dried on a country filter paper and transferred to 30 ml glass serum vials and sealed. By using a sterile syringe, 10 per cent of the air inside the bottle i.e. 3.0 ml was evacuated and replaced with 3.0 ml of pure acetylene. The bottles were incubated with 3000 lux light intensity for 20 hours at 28±1°C. One ml of gas mixture from the bottle was withdrawn and injected into

the Nucon 2865 Gas chromatograph. Peak height of ethylene was measured and recorded. Nitrogenase activity was calculated using the following formula.

$$\text{Nitrogenase activity} = \frac{\text{Peak height x Range x Attenuation x Volume of gas occupied x 0.001}}{\text{Dry weight of sample material x volume of gas injected x Hours of incubation.}}$$

The results were expressed as n mol of ethylene produced h^{-1} g^{-1} fresh weight.

3.5. MOLECULAR CHARACTERIZATION OF THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES

3.5.1. DNA extraction from the herbicide tolerant cyanobacterial isolates

The total DNA was extracted from the herbicide tolerant cyanobacterial isolates by following the method of Maniatis *et al.*(1982) .

Reagents

STE I buffer (pH 8.0)

100 mM NaCl	-	0.584 g
10 mM Tris	-	0.121 g
1 mM EDTA	-	0.037 g
Distilled water	-	100 ml

STE II buffer (pH 8.0)

100 mM NaCl	-	0.584 g
10 mM Tris	-	0.121 g
1 mM EDTA	-	0.93 g
Distilled water	-	100 ml

Phenol : Chloroform mixture : Phenol chloroform mixture was prepared in the ratio of 0.1 : 1.

Chloroform Isoamyl Alcohol mixture: Chloroform isoamyl alcohol mixture was prepared in the ratio of 24 : 1.

SDS 5% solution - freshly prepared solution was used

TE buffer (pH 8.0)

1 mM Tris	-	0.242 g
1 mM EDTA	-	0.074 g
Distilled water	-	200 ml

TBE buffer (10X)

0.9 M Tris	-	215.5 g
Boric Acid	-	110.06 g
0.5M EDTA	-	16.38 g
Distilled water	-	2000 ml

Procedure

The cyanobacterial isolates *viz.* *Anabaena*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria* -HT-SGK-1, *Westiellopsis*-HT-SGK-1, and *Westiellopsis*-HT-SGK-2 were grown in sterile N free BG-11 medium till the logarithmic growth phase. One ml of the culture suspension was transferred aseptically to a sterile 1.5 ml microfuge tube and centrifuged at 5000 rpm for 5 minutes. The cell pellet thus obtained was suspended in 1000 µl of STE 1 buffer. The cell suspension was then centrifuged at 10000 rpm for 6 minutes.

The supernatant was discarded and the cell pellet was then suspended in 600 µl of STE-II buffer and subjected to sonication under ice (3 pulses). The sonicated sample was immediately suspended in 60 µl of 5% SDS and kept at 70°C for 20 minutes.

To the lysed cell suspension, 600 µl of chloroform isoamyl alcohol mixture followed by 100 µl of phenol chloroform mixture were added and spun at 8000 rpm for 12 minutes. The aqueous phase was transferred to 600 µl of

chloroform isoamyl mixture in a fresh microfuge and spun at 8000 rpm for 10 minutes.

The aqueous phase was transferred to a fresh microfuge tube and 300 µl of isopropanol was added and spun at 8000 rpm for 12 minutes. The aqueous phase was decanted and 0.5 ml of icecold 70% alcohol was added and spun for 5 minutes at 4°C. The aqueous layer was removed and the pellet dried and suspended in 20 µl TE buffer. The agarose gel electrophoresis was performed for DNA analysis.

A 0.8% agarose gel was cast using TBE buffer and the samples were loaded on to the wells with a lambda DNA EcoRI-Hind III double digest as marker. The gel was stained with ethidium bromide and visualised under the UV illuminator for visualising the DNA and plasmids .

3.6. EFFECT OF INOCULATION OF HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES ON RICE SEEDLINGS

A pot culture experiment was conducted in plastic tubs (15 cm dia, 6 cm height) with three replications in a completely randomized design, replicated thrice. The tubs were filled with 2 kg sterile soil and pre germinated seeds of the rice variety ADT 36 were sown at the rate of 2.5 g per tub. Butachlor was applied at the rate of 30 µl per tub on 8th day after sowing. The herbicide tolerant cyanobacterial cultures *viz.*, *Anabaena*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria* -HT-SGK-1, *Westiellopsis* - HT-SGK-1, and *Westiellopsis*-HT-SGK-2 were inoculated individually and in combination at the rate of 1g of fresh weight per tub, 11 days after sowing. An uninoculated control was maintained for comparison. The water level was maintained at 2 cm throughout the study period. Plant height, ammonia excretion and total

chlorophyll in leaves were determined at 20 and 30 days after sowing. Total nitrogen was estimated at 30 days after sowing.

3.6.1. Plant height determination

The shoot growth (in cm) of rice seedlings was determined at 10 days interval from 10th day after sowing upto the 30th day.

3.6.2. Estimation of total chlorophyll

Total chlorophyll content of rice seedlings was estimated by the procedure given by Winterman and Demotes (1965). One gram of leaf sample was homogenized with 95 per cent ethanol and incubated in the dark at room temperature for 15 minutes. The extract was centrifuged at 5000 rpm for 5 minutes and the absorbance of the supernatant was read in a Beckman DU-64 spectrophotometer at 665 nm and 649 nm as against 95 per cent ethanol as blank. The total chlorophyll content was determined using the formula

$$\text{Total chlorophyll} = \frac{6.10\,(A_{665}) + 20.04\,(A_{640})}{\text{Volume of ethyl alcohol (ml)}}$$

The results were expressed in terms of mg g^{-1} fresh weight

3.6.3. Estimation of ammonia excretion pattern in flood water in soils planted with ADT 36 rice seedlings

Ammonia excretion by the herbicide tolerant cyanobacterial isolates inoculated to the rice seedlings was estimated by collecting the flood water samples at 10 days interval from 10th day of sowing by following the method of Solarzono (1969) as described earlier in section 3.3.3.

3.6.4. Estimation of total nitrogen in ADT 36 rice seedlings

The total nitrogen content in plant samples at 30 days after sowing was estimated by following the Kjeldahl method (Humpries, 1956).

Reagents

Catalyst mixture: $CuSO_4$ and K_2SO_4 in the ratio of 1:10 (w/w) .

Alkali solution : 40 per cent NaOH solution.

Double acid mixture: Conc. sulphuric acid and perchloric acid in the ratio of 8:2 (v:v)

Double Indicator : Bromocresol green - Methyl Red indicator

Boric acid- indicator mixture: Boric acid (20 g) was dissolved in 1 litre of water to which 20 ml of double indicator was added.

Procedure

Dried and powdered plant sample (0.5 g) was transferred to a dry digestion tube of VELP SCIENTIFIC UDK 126 digester. Ten ml of double acid was added and the contents were thoroughly mixed and allowed to stand for 30 minutes. Then 0.5 g of catalyst mixture was added to the tubes and heated slowly till frothing continued and then digested for 1 h. The tubes were then cooled and 100 ml of distilled water was added slowly and this mixture was used for the determination of total nitrogen. The nitrogen present was estimated in a VELP SCIENTIFICA UDK 126 (semi autoanalyser) after adding approximately 100 ml of 40 per cent sodium hydroxide. The ammonia liberated was collected in boric acid indicator solution and titrated against 0.5 N sulphuric acid. From the titre value the nitrogen content was calculated using the formula.

$$\text{Nitrogen content \%} = \frac{a \times 0.00028 \times V \times 100}{10 \times W}$$

a - Volume of N/50 H_2SO_4 used (ml)

V - Volume of diacid extract prepared (ml)

10 - Volume of diacid extract used for analysis

W - Weight of the samples

The results were expressed as percentage of total nitrogen present.

3.7. STUDIES ON THE EFFECT OF INOCULATION OF HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES ALONG WITH GRADED LEVELS OF NITROGEN ON THE YIELD OF ADT 36 RICE

A pot culture experiment was conducted in a completely randomized block design in cement tubs (1 m^2) with 10 treatments and 3 replications. The tubs were filled with 300 kg of clayey loam soil and ADT 36 rice seedlings (25 days old) were transplanted in the tubs with a spacing of 15 x 10 cm. Butachlor was applied at the rate of 3 ml to the selected treatments 3 days after transplanting. 5.0 g of fresh composite culture was applied as biofertilizer at 10 days after transplanting. A fertilizer regime of 120 : 50 : 50 kg N : P : K was followed, Nitrogen was applied as urea in four split doses *viz.*, basal, active tillering, flowering and milking stages to the selected treatments. Phosphorus as super phosphate and potassium as muriate of potash were applied in the full recommended doses to all the treatments basally. The water level was maintained at 5 cm throughout the study period. The treatments were

T_1 - No herbicide, no inoculation and no nitrogen (H_0 I_0 N_0)
T_2 - No herbicide, inoculation and no nitrogen (H_0 I_1 N_0)
T_3 - Herbicide, no inoculation and no nitrogen (H_1 I_0 N_0)
T_4 - Herbicide, inoculation and no nitrogen (H_1 I_1 N_0)
T_5 - No herbicide, no inoculation and 75% nitrogen (H_0 I_0 N_{75})
T_6 - No herbicide, inoculation and 75% nitrogen (H_0 I_1 N_{75})
T_7 - Herbicide, no inoculation and 75% nitrogen (H_1 I_0 N_{75})
T_8 - Herbicide, inoculation and 75% nitrogen (H_1 I_1 N_{75})
T_9 - Herbicide, no inoculation and 100% nitrogen (H_1 I_0 N_{100})
T_{10} - Herbicide, inoculation and 100% nitrogen (H_1 I_0 N_{100})
H_0 - No herbicide
H_1 - Herbicide
N_0 - No Nitrogen
I_0 - No inoculation
I_1 - Inoculation

3.7.1. Plant height determination

The plant height (cm) of the rice plants was determined at intervals of 30 days upto harvest of the paddy crop.

3.7.2. Estimation of yield attributes

Individual yield attributes *viz.*, total tillers hill^{-1},productive tillers hill^{-1}, panicle length,number of filled grains panicle^{-1} and 1000 grain weight were recorded.

3.7.3. Estimation of total nitrogen and nitrogen uptake by rice plant

Total nitrogen present in the soil samples was estimated by following the Kjeldhal method (Humpries, 1956) as described earlier in section 3.6. Total nitrogen was expressed as percentage. Nitrogen uptake was estimated by following the formula and expressed as gm^{-2}.

$$\text{N uptake } (gm^{-2}) = \frac{\text{Nitrogen content x Dry matter } (g\ m^{-2})}{100}$$

3.8. STATISTICAL ANALYSIS

The data were subjected to statistical scrutiny as per the methods detailed by Panse and Sukhatme (1985).

RESULTS

4. EXPERIMENTAL RESULTS

4.1. ISOLATION AND PURIFICATION OF CYANOBACTERIA FROM HERBICIDE AMENDED SOILS

Isolation of cyanobacteria was done from soils amended with the herbicide. From the data given in Table 1 it could be concluded that the dominant genera was *Westiellopsis* (46.0%), followed by *Anabaena* (23.0%), *Nostoc* (22.3%) and *Oscillatoria* (7.0%). The cyanobacterial isolates were purified by growing them in BG-11 medium using the triple antibiotic solution. Plate 1 shows the streak plate method of purifying the cyanobacterial isolates. Plates 2 to 7 shows the unialgal cultures of the selected cyanobacterial isolates *viz.*, *Anabeana*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria*-HT-SGK-1, *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 isolated from the herbicide amended rice soils.

4.2. GROWTH PERFORMANCE AND BIOCHEMICAL CONSTITUENTS OF THE CYANOBACTERIAL ISOLATES FROM HERBICIDE AMENDED SOILS

4.2.1. Growth and biomass production by the cyanobacterial isolates from herbicide amended soils

The growth and biomass production of the cyanobacterial isolates from herbicide amended soil were observed at 21 days after inoculation and the results are presented in Tables 2 and 3 and Fig. 1. Among the cyanobacterial isolates *Westiellopsis*-HT-SGK-2 recorded maximum growth and biomass production followed by *Westiellopsis*-HT-SGK-1. The cyanobacterial isolate *Anabaena*-HT-SGK-1 recorded the lowest growth and biomass production.

4.2.2. Chlorophyll content of the cyanobacterial isolates from herbicide amended soils

The results are presented in Table 4 and Fig. 2. The cyanobacterial isolates *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 recorded significantly

Table 1. Cyanobacterial genera isolated from herbicide amended soils

Sample No.	Treatment	Genera	Code
1	Butachlor + Handweeding	*Westiellopsis*	4
		Westiellopsis	5
		Anabaena	1
		Anabaena	3
2	2,4-D + Handweeding	*Westiellopsis*	3
		Nostoc	1
		Oscillatoria	1
3	Butachlor + 2,4-D	*Westiellopsis*	2
		Nostoc	1
		Anabaena	2
4.	Butachlor	*Westiellopsis*	1
		Westiellopsis	6
		Nostoc	2

Plate 1. **Purification of herbicide tolerant cyanobacterial isolates**

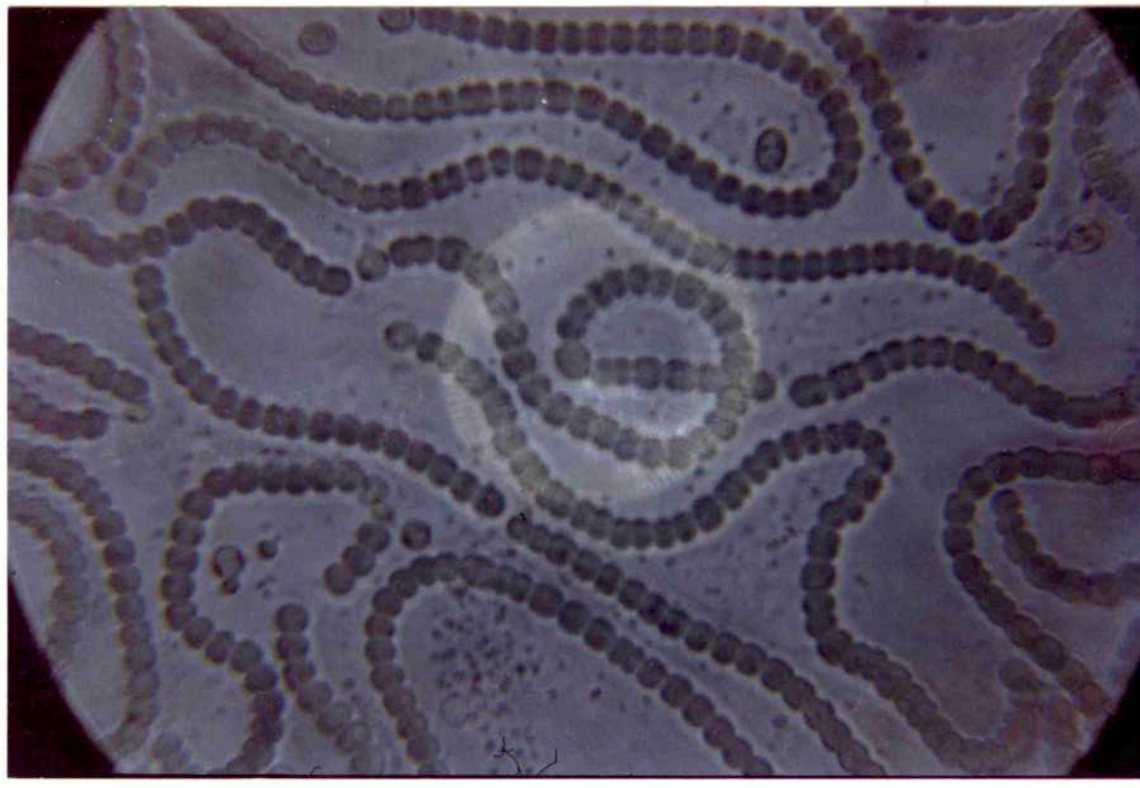


Plate 2. **Microscopic view of *Anabaena* - HT-SGK-1**

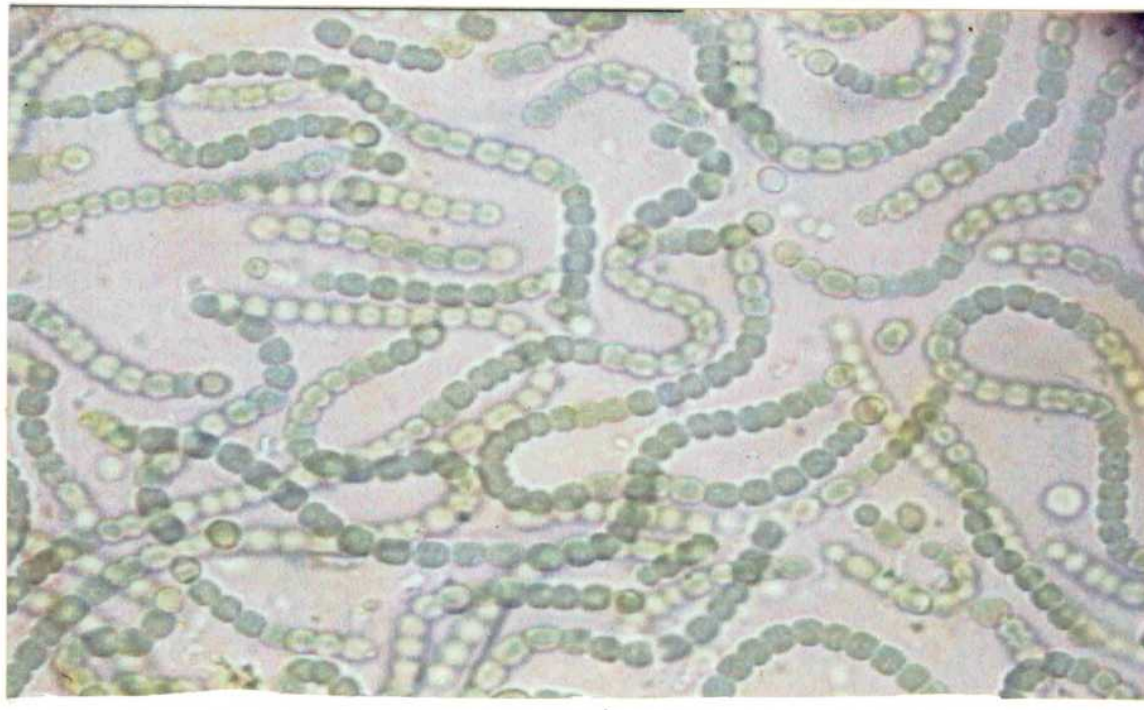


Plate 3. ***Anabaena* - HT-SGK-2**

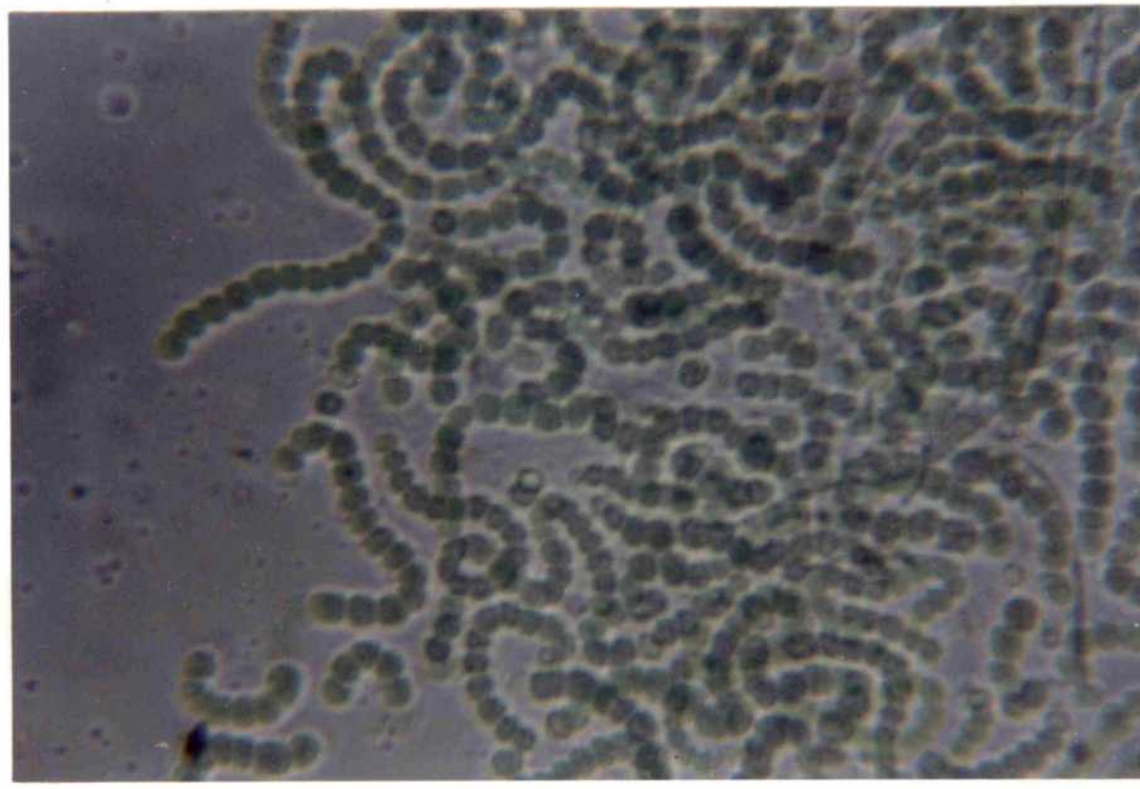


Plate 4. *Nostoc* - **HT-SGK-1**

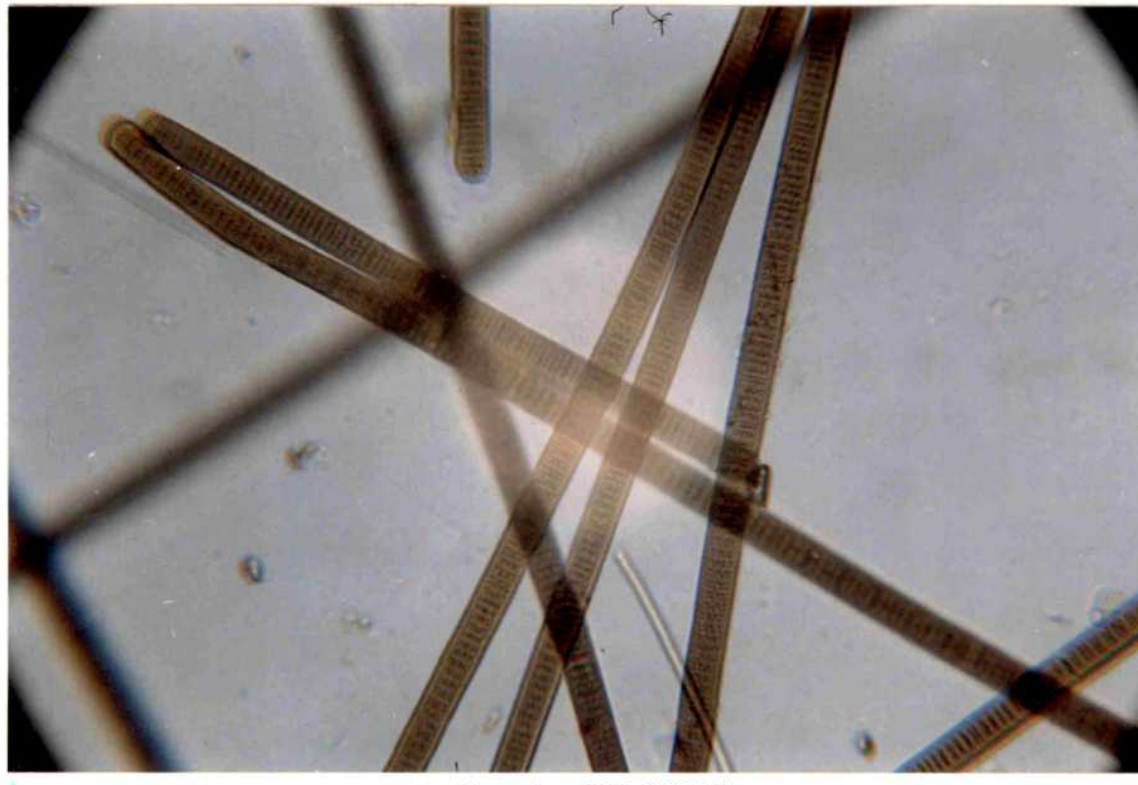


Plate 5. *Oscillatoria* - **HT-SGK-1**

Plate 6. ***Westiellopsis*** **- HT-SGK-1**

Plate 7. ***Westiellopsis*** **- HT-SGK-2**

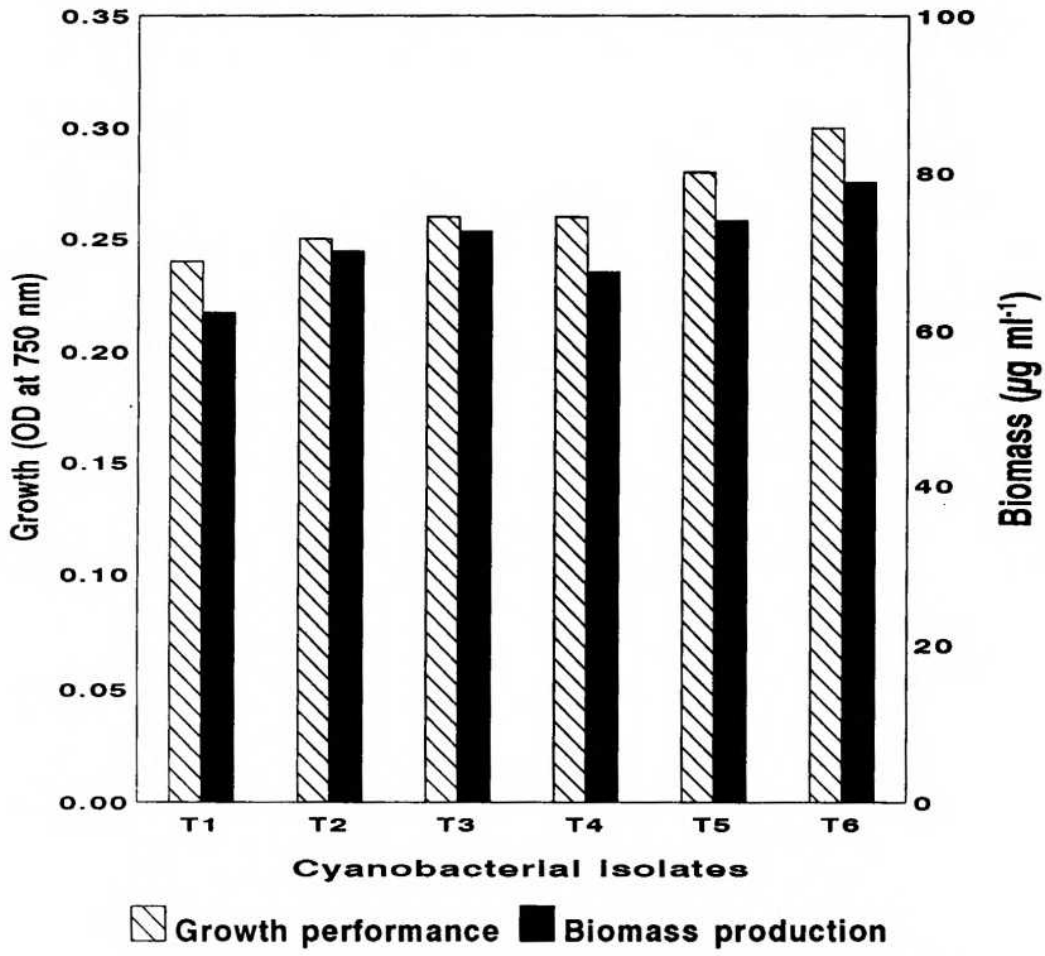
Table 2. Growth performance of the cyanobacterial isolates from herbicide amended soils.

Isolates	Growth (0D at 750 nm)
Anabaena-HT-SGK-1	0.24
Anabaena--HT-SGK-2	0.25
Nostoc-HT-SGK-1	0.26
Oscillatoria-HT-SGK-1	0.26
Westiellopsis -HT-SGK-1	0.28
Westiellopsis-HT-SGK-2	0.30
SEd	0.01
CD	0.03

Table 3. Biomass production by the cyanobacterial isolates from herbicide amended soils.

Isolates	Biomass (μg ml $^{-1}$)
Anabaena-HT-SGK-1	62.06
Anabaena--HT-SGK-2	69.82
Nostoc-HT-SGK-1	72.40
Oscillatoria-HT-SGK-1	67.23
Westiellopsis -HT-SGK-1	73.80
Westiellopsis-HT-SGK-2	78.69
SEd	2.67
CD	5.82

Fig 1. Growth and biomass production of cyanobacterial isolates from herbicide amended soils

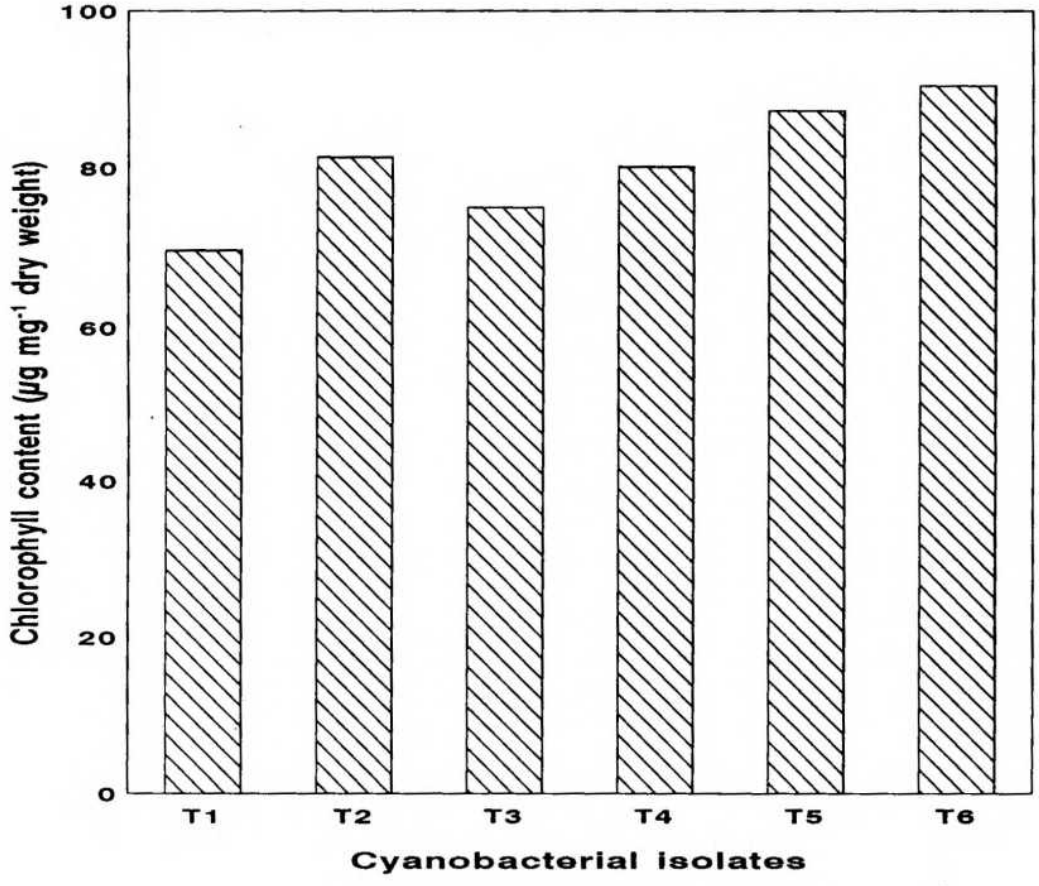


T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 4. Chlorophyll-a content of the cyanobacterial isolates from herbicide amended soils.

Isolates	Chlorophyll-a content ($\mu g\ mg^{-1}$ dry weight)
Anabaena-HT-SGK-1	69.56
Anabaena--HT-SGK-2	81.30
Nostoc-HT-SGK-1	74.97
Oscillatoria-HT-SGK-1	80.08
Westiellopsis -HT-SGK-1	87.20
Westiellopsis-HT-SGK-2	90.38
SEd	3.38
CD	7.37

Fig 2. Chlorophyll-a content of the cyanobacterial isolates from herbicide amended soils



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

higher chlorophyll content. Though *Nostoc*-HT-SGK-1 registered higher chlorophyll content, it was on par with *Oscillatoria*-HT-SGK-1 and *Anabaena*-HT-SGK-2. *Anabaena*-HT-SGK-1 recorded significantly lowest chlorophyll content.

4.2.3. Ammonia excretion by the cyanobacterial isolates from herbicide amended soils

The ammonia excretion by the cyanobacterial isolates was assessed and the results are presented in Table 5 and Fig. 3. Among the isolates *Westiellopsis*-HT-SGK-2 excreted significantly higher amount of ammonia into the growth medium followed by *Westiellopsis*-HT-SGK-1. *Anabaena*-HT-SGK-1 excreted the lowest level of ammonia.

4.2.4. C-phycocyanin content of the cyanobacterial isolates from herbicide amended soils

The results are given in Table 6 and Fig.4 Among the isolates *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 have recorded significantly higher C-phycocyanin content. Though *Nostoc*-HT-SGK-1 has registered higher C-phycocyanin content, it was on par with *Oscillatoria*-HT-SGK-1 and *Anabaena*-HT-SGK-2. The isolate *Anabaena*-HT-SGK-1 recorded the lowest C-phycocyanin content.

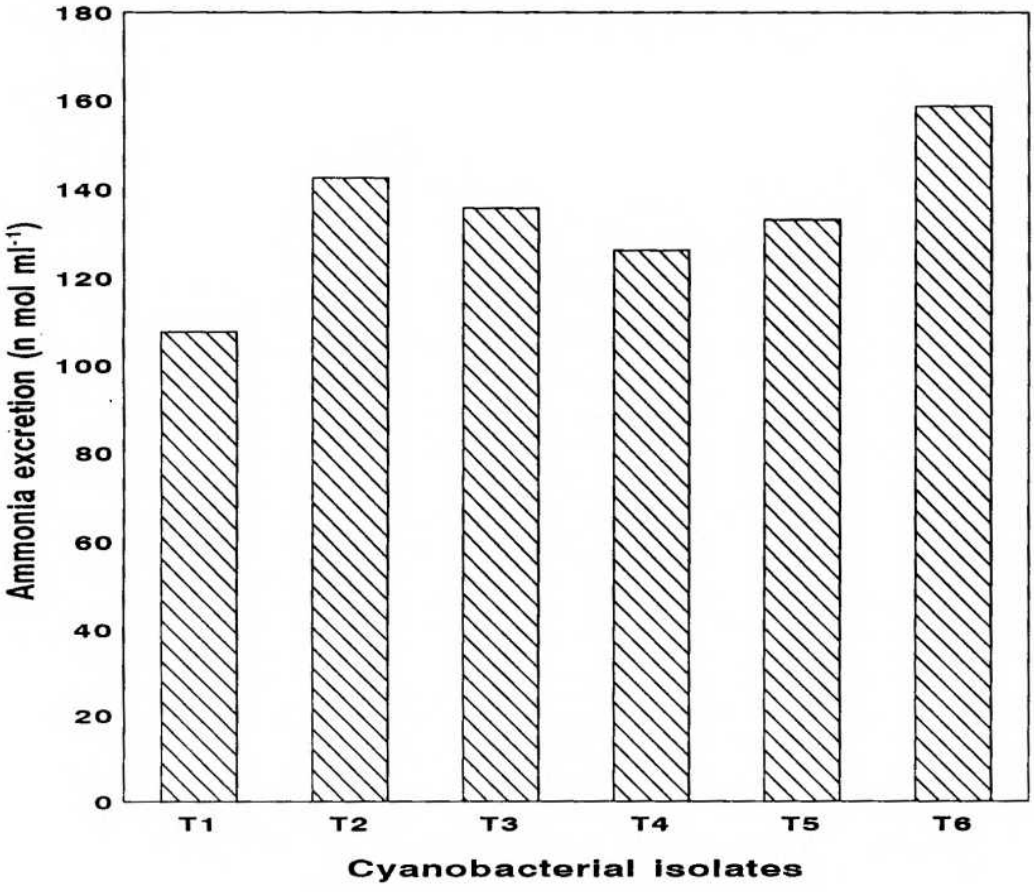
4.2.5. C-phycoerythrin content of the cyanobacterial isolates from herbicide amended soils

The results are presented in Table 7 and Fig.4. Among the isolates *Westiellopsis*-HT-SGK-2 recorded significantly higher C-phycoerythrin content followed by *Westiellopsis*-HT-SGK-1 which was on par with *Nostoc*-HT-SGK-1. Between the *Anabaena* isolates *Anabaena*-HT-SGK-2 registered higher C-phycoerythrin content. While *Anabaena*-HT-SGK-1 registered significantly lower C- phycoerythrin content.

Table 5. Ammonia excretion by the cyanobacterial isolates from herbicide amended soils.

Isolates	Ammonia excretion (n mol ml^{-1})
Anabaena-HT-SGK-1	107.56
Anabaena--HT-SGK-2	142.48
Nostoc-HT-SGK-1	135.66
Oscillatoria-HT-SGK-1	129.20
Westiellopsis -HT-SGK-1	133.04
Westiellopsis-HT-SGK-2	158.59
SEd	5.83
CD	12.70

Fig 3. Ammonia excretion by cyanobacterial isolates from herbicide amended soils



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 6. C-phycocyanin content of the cyanobacterial isolates from herbicide amended soils.

Isolates	C-phycocyanin ($\mu g\ mg^{-1}$ dry weight)
Anabaena-HT-SGK-1	5.38
Anabaena--HT-SGK-2	6.80
Nostoc-HT-SGK-1	7.67
Oscillatoria-HT-SGK-1	7.16
Westiellopsis -HT-SGK-1	11.03
Westiellopsis-HT-SGK-2	11.04
SEd	0.34
CD	0.76

Table 7. C- phycoerythrin content of the cyanobacterial isolates from herbicide amended soils.

Isolates	C- phycoerythrin content ($\mu g\ mg^{-1}$ dry weight)
Anabaena-HT-SGK-1	5.77
Anabaena--HT-SGK-2	6.87
Nostoc-HT-SGK-1	8.85
Oscillatoria-HT-SGK-1	8.24
Westiellopsis -HT-SGK-1	9.16
Westiellopsis-HT-SGK-2	10.00
SEd	0.33
CD	0.73

4.2.6. Allophycocyanin content of the cyanobacterial isolates from herbicide amended soils

The results are presented in Table 8 and Fig.4. Maximum allophycocyanin content was noticed in *Westiellopsis*-HT-SGK-2 followed by *Westiellopsis*-HT-SGK-1. *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1 were on par with each other. Between the *Anabaena* isolates maximum allophycocyanin was recorded by *Anabaena*-HT-SGK-2.

4.2.7. Protein content of the cyanobacterial isolates from herbicide amended soils

The results are presented in Table 9 and Fig.5. The protein content was significantly higher in *Westiellopsis*-HT-SGK-2 followed by *Westiellopsis*-HT-SGK-1. The protein content was on par with *Anabaena*-HT-SGK-2. *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1 while *Anabaena*-HT-SGK-1 registered significantly lowest protein content.

4.3. HERBICIDE TOLERANCE STUDIES

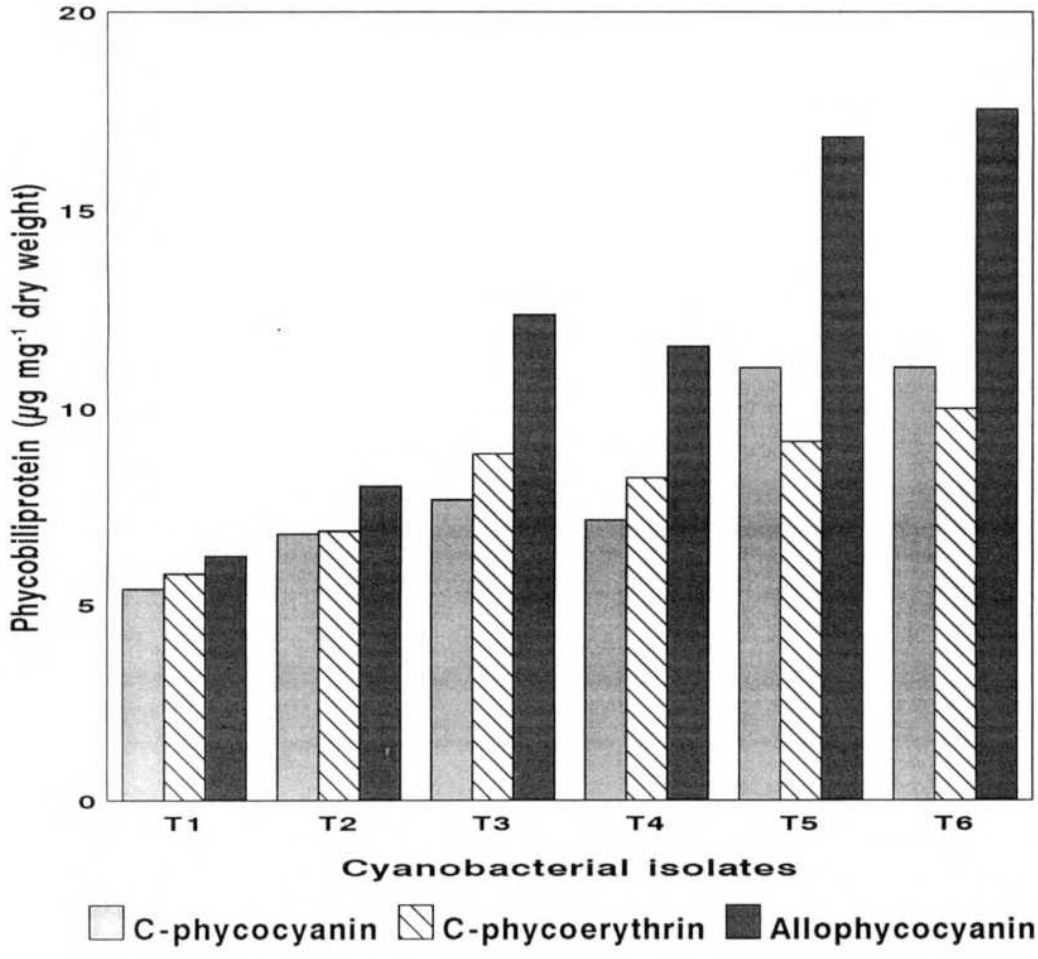
4.3.1. Effect of butachlor on the growth and biomass production of the herbicide tolerant cyanobacterial isolates

The results are presented in Tables 10 and 11 and Figs.6 and 7 and Plates 8 to 13. The growth and biomass production showed a linear increase corresponding to the incubation period. However the inhibitory effect of butachlor on cyanobacterial isolates was noticed on the 10^{th} day only. Among the isolates, *Westiellopsis*-HT-SGK-2 and *Westiellopsis*-HT-SGK-1 recorded maximum growth and biomass at all the concentrations of butachlor. Interestingly the herbicide butachlor was found to be stimulatory to *Westiellopsis* cultures and inhibitory to the other cultures. Marked reduction was noticed at 12ppm concentration on the 30^{th} day in the case of *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1, while no reduction was noticed in the growth and biomass production of *Anabaena* cultures

Table 8. Allophycocyanin content of the cyanobacterial isolates from herbicide amended soils.

Isolates	Allophycocyanin content ($\mu g\ mg^{-1}$ dry weight)
Anabaena-HT-SGK-1	6.22
Anabaena--HT-SGK-2	8.02
Nostoc-HT-SGK-1	12.37
Oscillatoria-HT-SGK-1	11.57
Westiellopsis -HT-SGK-1	16.83
Westiellopsis-HT-SGK-2	17.54
SEd	3.38
CD	7.37

Fig 4. Phycobiliprotein content of cyanobacterial isolates from herbicide amended soils

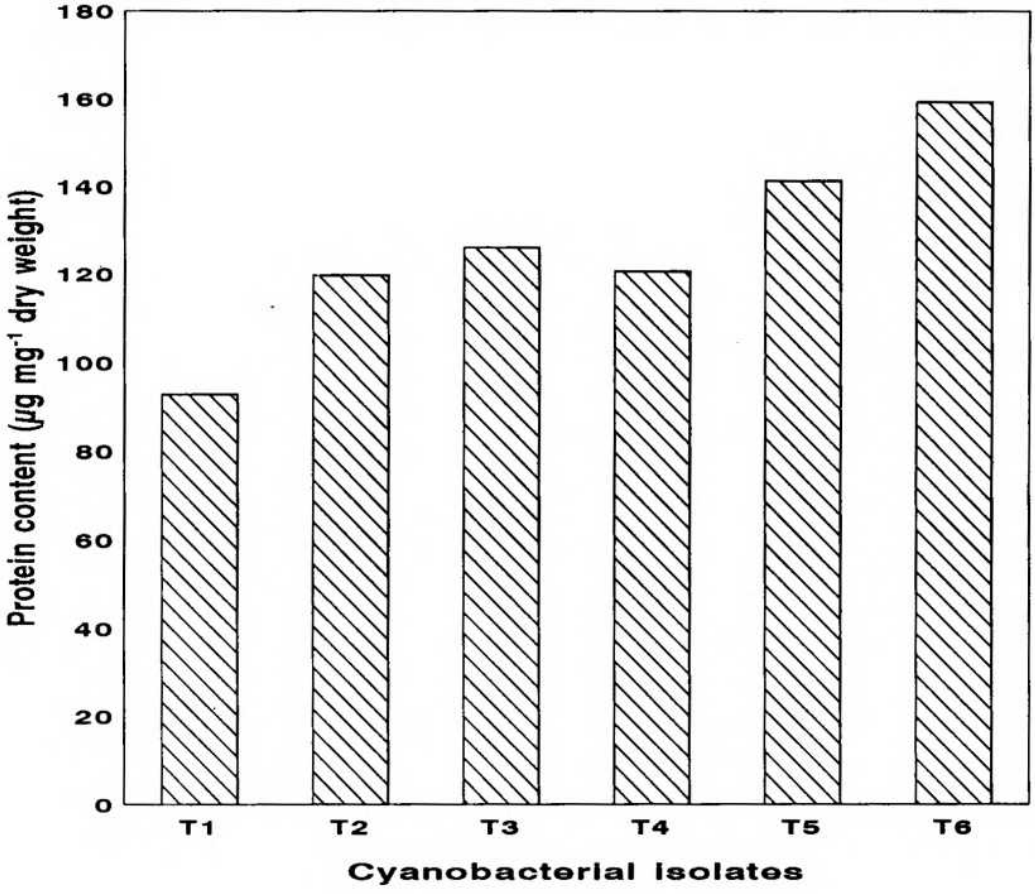


T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 9. Protein content of the cyanobacterial isolates from herbicide amended soils.

Isolates	Protein content ($\mu g\ mg^{-1}$ dry weight)
Anabaena-HT-SGK-1	92.74
Anabaena--HT-SGK-2	119.65
Nostoc-HT-SGK-1	126.05
Oscillatoria-HT-SGK-1	120.52
Westiellopsis -HT-SGK-1	141.17
Westiellopsis-HT-SGK-2	159.21
SEd	5.16
CD	11.25

Fig 5. Protein content of cyanobacterial isolates from herbicide amended soils



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 10. Effect of butachlor on the growth (OD at 750 nm) of the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	0.12	0.25	0.36
	3	0.08	0.22	0.31
	6	0.07	0.19	0.30
	9	0.04	0.17	0.27
	12	0.03	0.16	0.25
Anabaena-HT-SGK-2	0	0.09	0.33	0.52
	3	0.10	0.33	0.62
	6	0.13	0.36	0.50
	9	0.13	0.49	0.59
	12	0.09	0.21	0.48
Nostoc-HT-SGK-I	0	0.12	0.28	0.44
	3	0.10	0.24	0.37
	6	0.09	0.21	0.33
	9	0.08	0.18	0.28
	12	0.06	0.14	0.22
Oscillatoria-HT-SGK-I	0	0.09	0.21	0.49
	3	0.09	0.20	0.46
	6	0.08	0.21	0.30
	9	0.07	0.21	0.30
	12	0.05	0.11	0.25
Westiellopsis-HT-SGK-I	0	0.11	0.32	0.49
	3	0.13	0.32	0.49
	6	0.11	0.30	0.55
	9	0.13	0.35	0.54
	12	0.13	0.28	0.63
Westiellopsis-HT-SGK-2	0	0.09	0.32	0.51
	3	0.11	0.30	0.50
	6	0.09	0.29	0.52
	9	0.10	0.31	0.56
	12	0.11	0.30	0.51

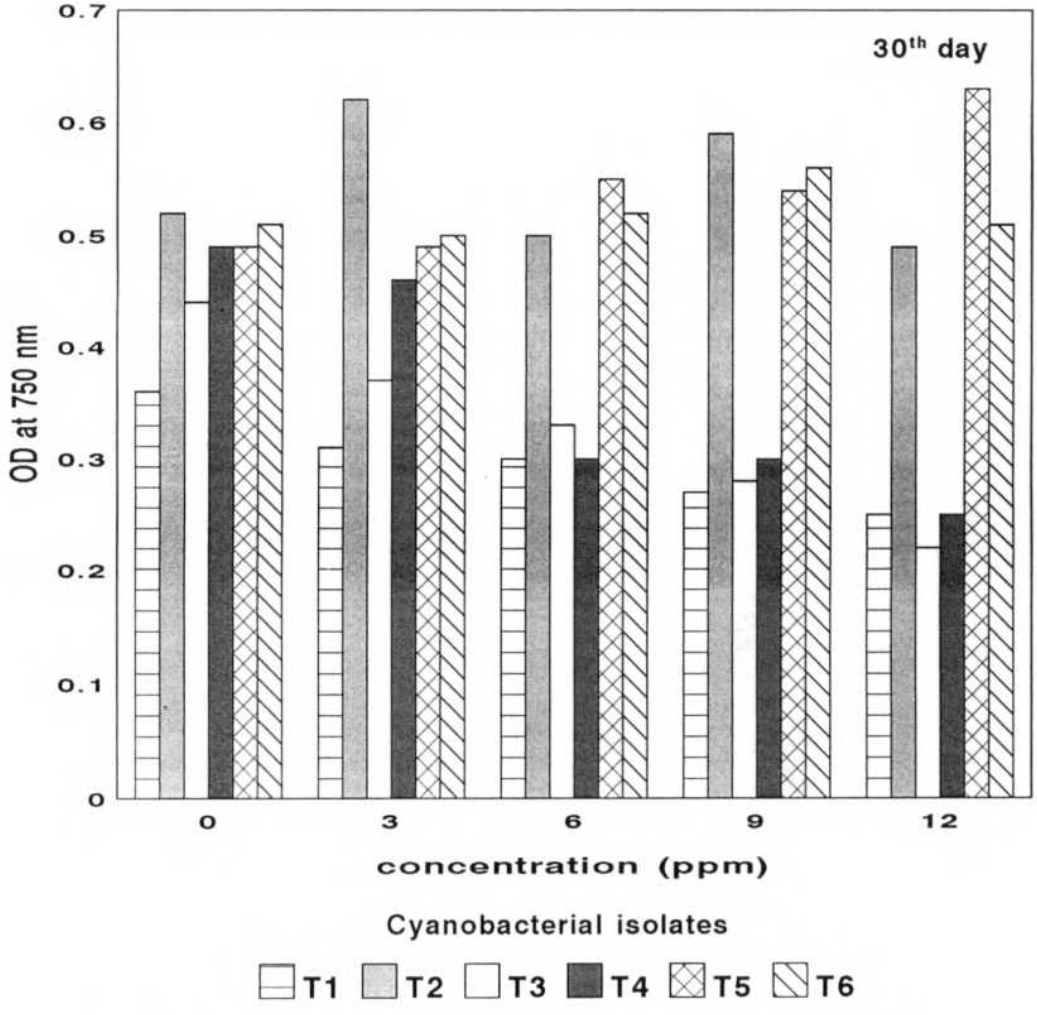
	SEd	CD
Cultures	0.007	0.15
Concentration	0.007	0.13
Days	0.17	0.34
Cultures x Concentration	0.12	0.24
Cultures x Concentration x Days	0.03	0.05

Table 11. Effect of butachlor on the biomass production (µg ml^{-1}) by the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	24.57	51.19	73.71
	3	16.38	45.04	63.47
	6	14.33	38.90	61.42
	9	8.19	34.80	55.28
	12	6.14	32.16	51.90
Anabaena-HT-SGK-2	0	18.42	67.50	106.47
	3	20.47	66.53	126.94
	6	26.61	73.71	102.37
	9	24.37	83.94	120.80
	12	18.10	59.37	98.28
Nostoc-HT-SGK-I	0	30.54	71.27	112.00
	3	25.10	61.09	94.18
	6	22.90	53.45	84.00
	9	20.36	45.81	71.27
	12	15.37	35.63	56.00
Oscillatoria-HT-SGK-I	0	21.90	53.45	124.72
	3	20.09	50.92	117.09
	6	19.36	51.45	94.18
	9	17.80	51.09	70.36
	12	12.72	28.00	63.63
Westiellopsis-HT-SGK-I	0	28.00	73.50	140.00
	3	33.09	81.45	132.70
	6	28.00	76.36	140.00
	9	29.10	89.04	137.40
	12	28.20	71.27	160.30
Westiellopsis-HT-SGK-2	0	22.90	81.45	128.50
	3	28.00	76.36	127.21
	6	22.90	73.81	127.27
	9	25.45	78.90	132.36
	12	28.45	76.36	129.81

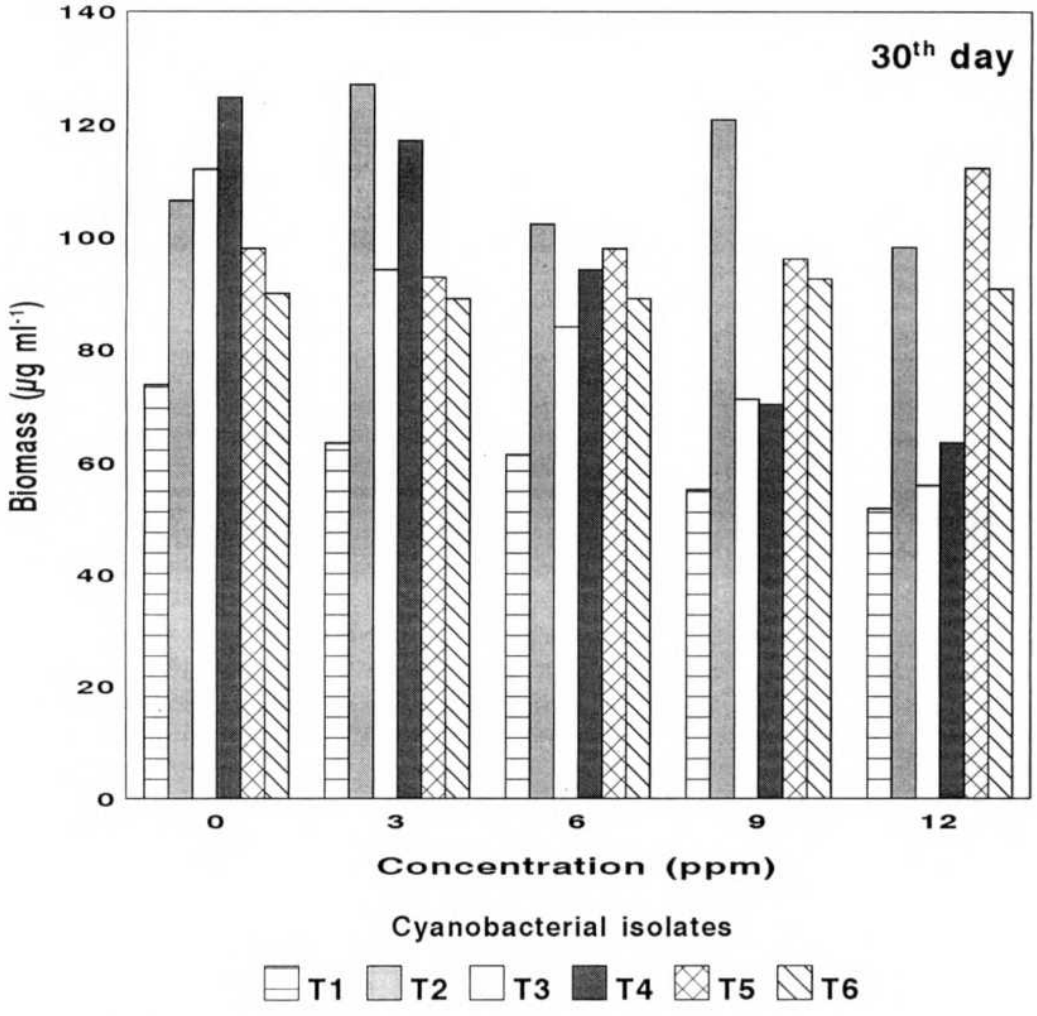
	SEd	CD
Cultures	1.96	3.88
Concentration	1.79	3.54
Days	1.39	2.74
Cultures x Concentration	3.11	6.14
Cultures x Concentration x Days	7.62	15.04

Fig 6. Effect of butachlor on the growth performance of the herbicide tolerant cyanobacterial isolates



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westiello*psis-HT-SGK-2

Fig 7. Effect of butachlor on the biomass production by the herbicide tolerant cyanobacterial isolates



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westiello*psis-HT-SGK-2

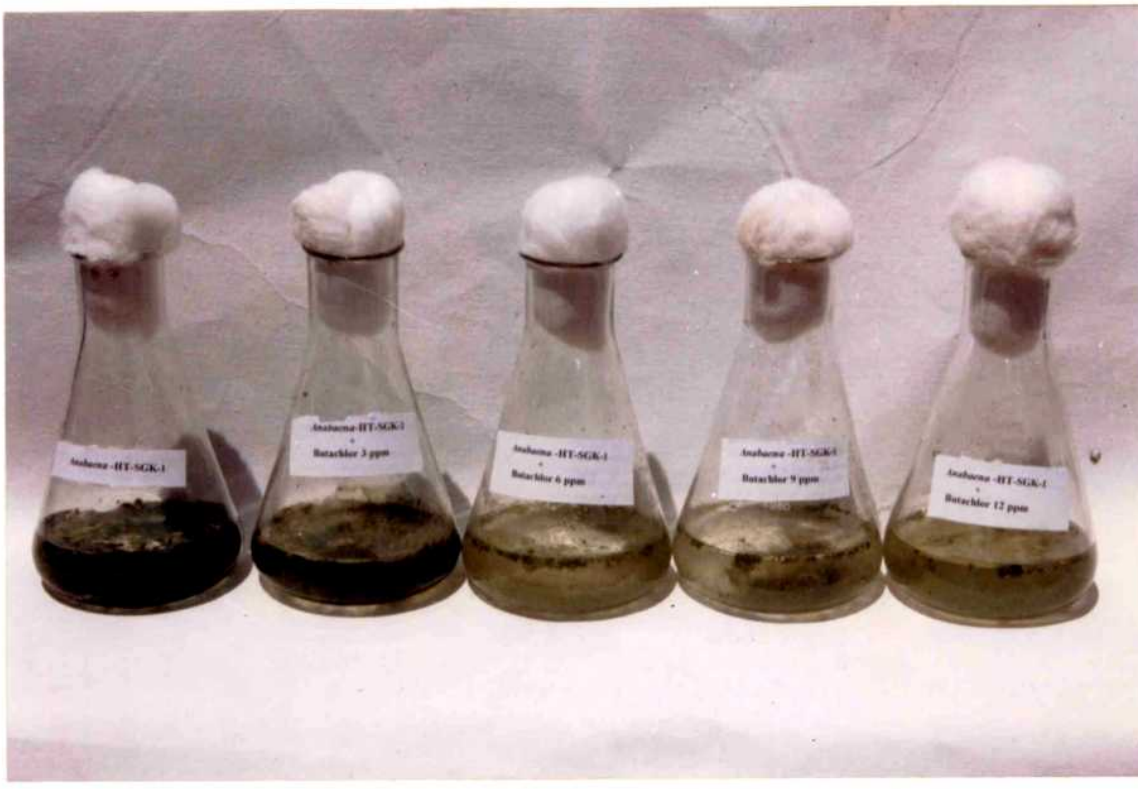


Plate 8. **Effect of butachlor on the growth of *Anabaena*-HT-SGK-1**

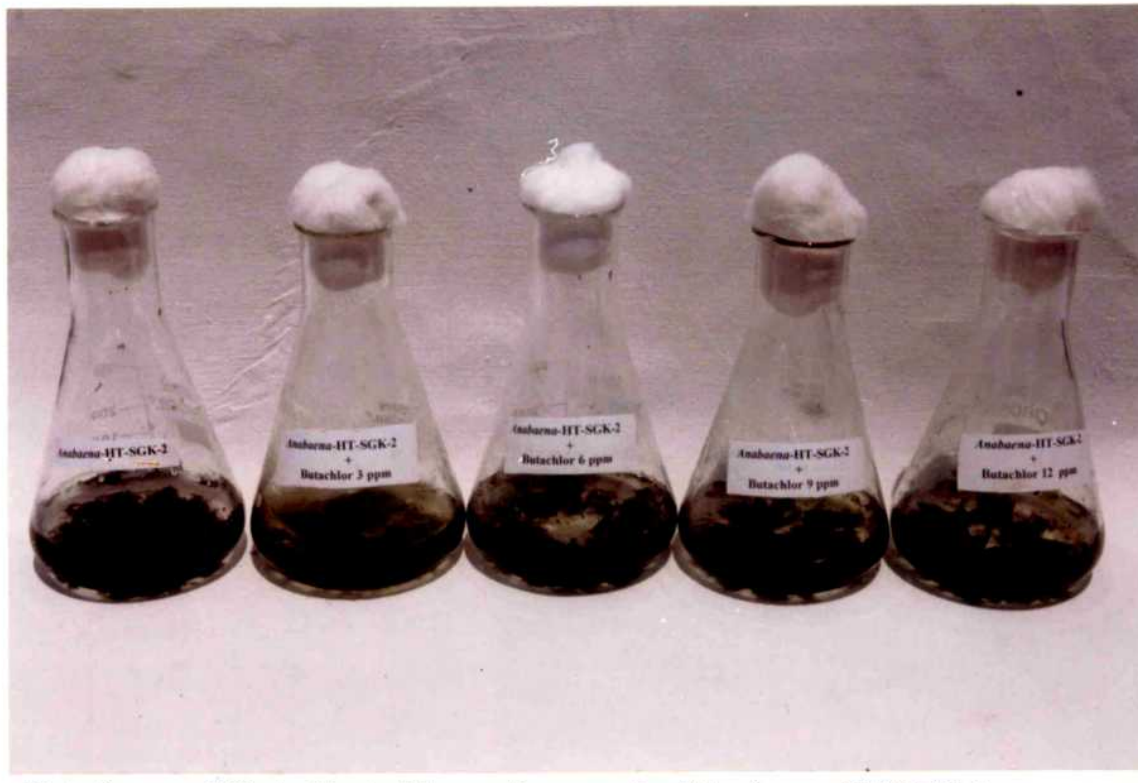


Plate 9. **Effect of butachlor on the growth of *Anabaena*-HT-SGK-2**

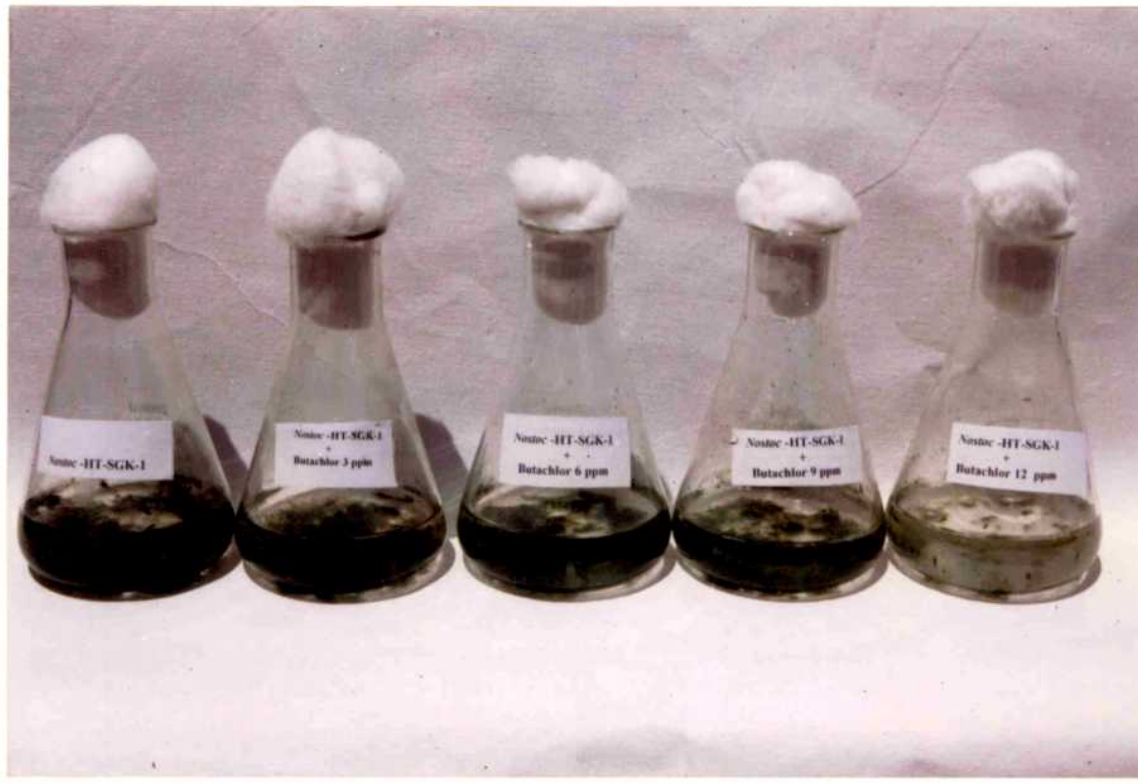


Plate 10. **Effect of butachlor on the growth of *Nostoc*-HT-SGK-1**

Plate 11. **Effect of butachlor on the growth of *Oscillatoria*-HT-SGK-1**

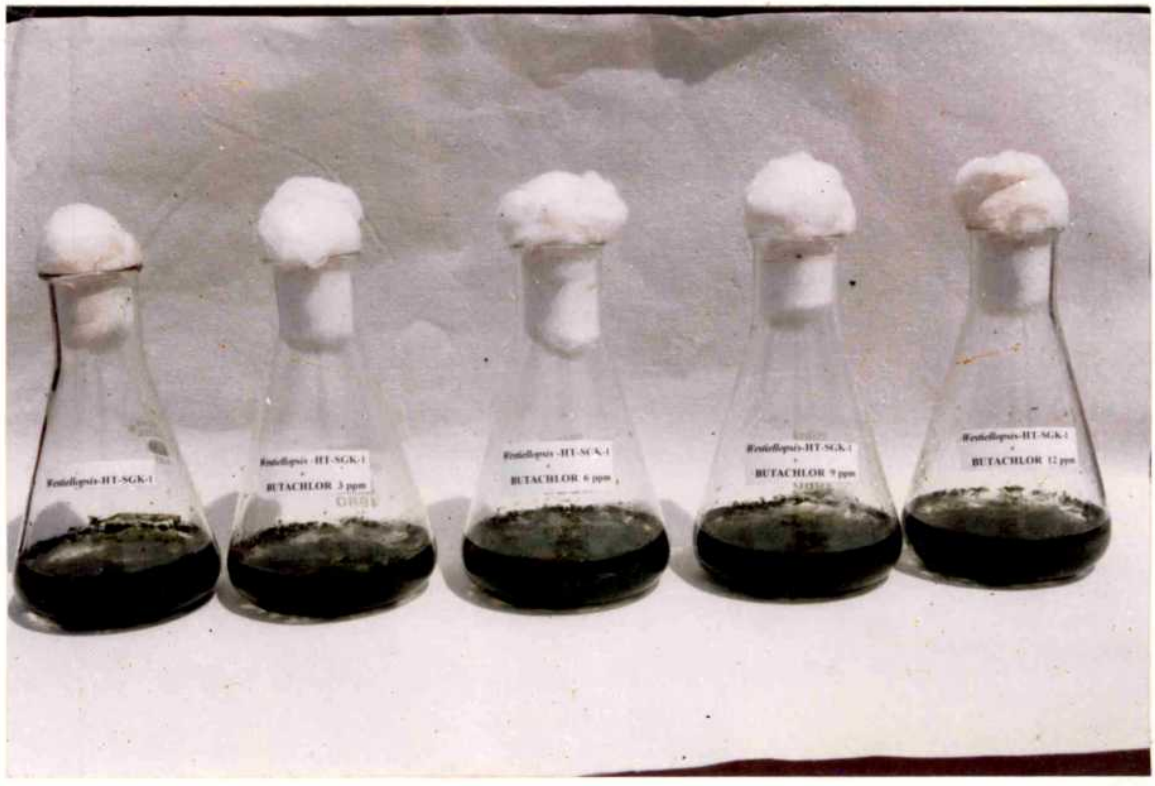


Plate 12. **Effect of butachlor on the growth of *Westiellopsis*-HT-SGK-1**

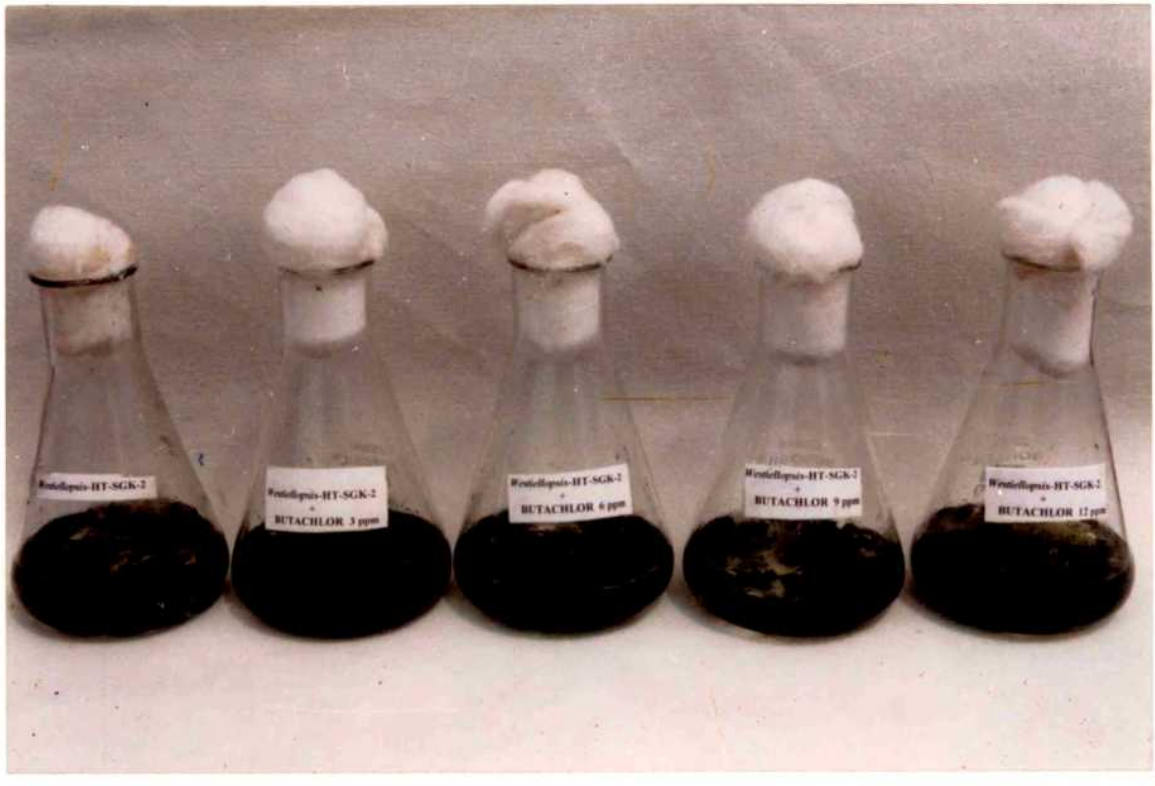


Plate 13. **Effect of butachlor on the growth of *Westiellopsis*-HT-SGK-2**

upto 6 ppm concentration. The results clearly indicated the ability of *Westiellopsis* cultures to tolerate higher concentrations of butachlor without any significant reduction in the growth and biomass production.

4.3.2. Effect of butachlor on the chlorophyll content of the herbicide tolerant cyanobacterial isolates

The results are presented in Table 12 and Fig.8. There was a linear increase in the chlorophyll content irrespective of the culture tested. However butachlor was found to be inhibitory at all the concentrations tested in the case of *Anabaena*-HT-SGK-1, *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1. Butachlor was found to be stimulatory to *Anabaena* upto 9ppm concentration at all the incubation periods. Interestingly *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 were found to tolerate butachlor upto 12ppm. The results clearly indicated that *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 were found to be the most herbicide tolerant cyanobacterial isolates.

4.3.3. Effect of butachlor on the ammonia excretion by the herbicide tolerant cyanobacterial isolates

The results are presented in Table 13 and Fig.9. Maximum ammonia excretion was noticed on the 10^{th} day. Among the cultures *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 showed higher ammonia excretion at all the incubation periods at all the herbicide concentrations. The ammonia excretion was drastically affected in *Oscillatoria*-HT-SGK-1 and *Nostoc*-HT-SGK-1 at 6 ppm concentration of butachlor. The cyanobacterial culture *Anabaena*-HT-SGK-1 registered lowest ammonia excretion at 30^{th} day at 12 ppm concentration.

4.3.4. Effect of butachlor on the C- phycocyanin content of the herbicide tolerant cyanobacterial isolates

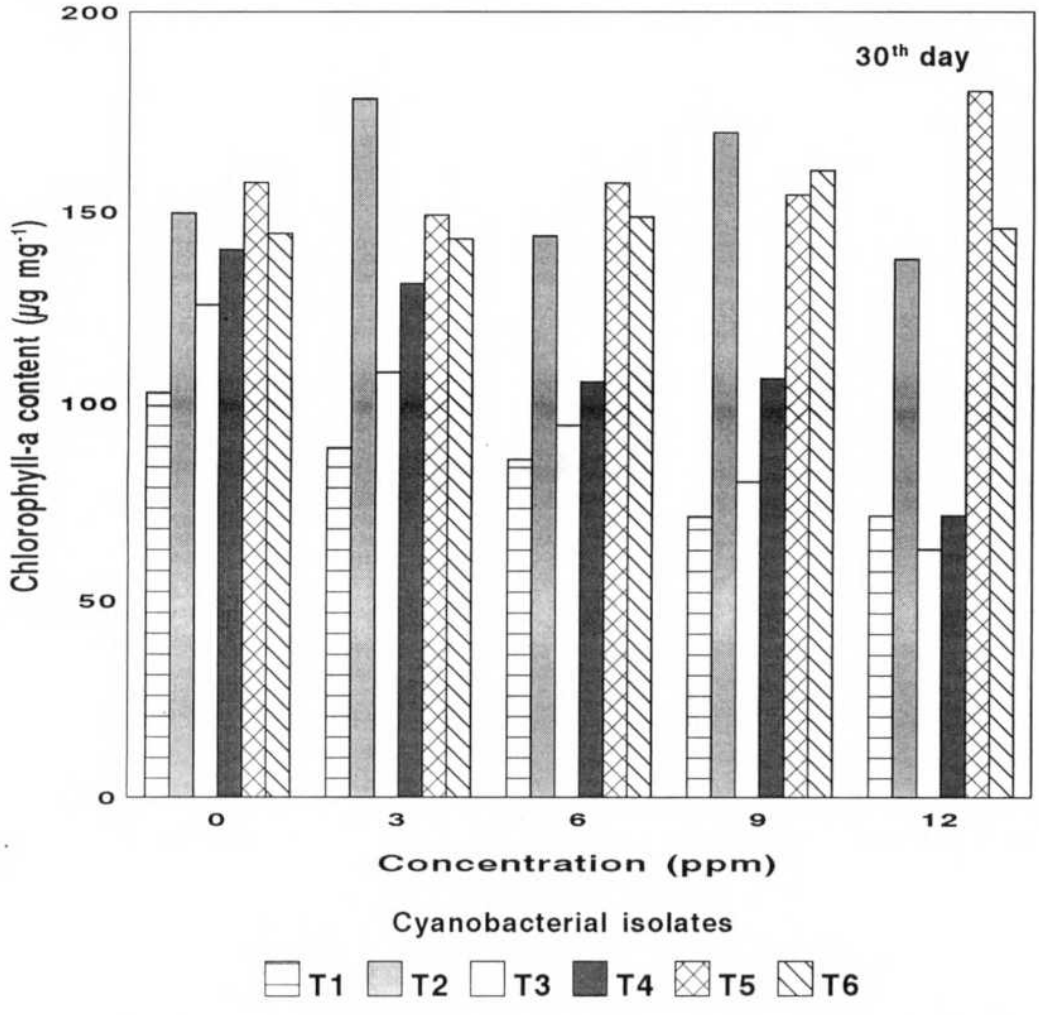
The results are presented in Table 14 and Fig.10. On the 10th day of observation, butachlor at 3 ppm was found to reduce the C -phycocyanin content of

Table 12. Effect of butachlor on chlorophyll content (µg mg^{-1} dry weight) of the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	34.43	71.42	102.85
	3	22.95	62.85	88.57
	6	20.00	54.28	85.71
	9	11.12	48.57	71.14
	12	8.57	45.71	71.42
Anabaena-HT-SGK-2	0	25.86	94.80	149.49
	3	28.74	93.41	178.23
	6	37.31	103.41	143.73
	9	34.20	117.85	169.96
	12	25.40	83.86	137.99
Nostoc-HT-SGK-I	0	34.32	80.08	125.84
	3	28.30	68.64	108.32
	6	25.64	60.16	94.38
	9	22.78	50.64	80.08
	12	17.16	39.04	62.92
Oscillatoria-HT-SGK-I	0	24.73	60.06	140.14
	3	25.74	56.20	131.56
	6	22.88	59.54	105.82
	9	20.02	59.44	106.82
	12	14.30	31.46	71.50
Westiellopsis-HT-SGK-I	0	31.46	82.50	157.32
	3	37.18	91.50	149.10
	6	31.46	85.90	157.30
	9	32.69	100.09	154.37
	12	31.68	80.07	180.10
Westiellopsis-HT-SGK-2	0	25.72	91.51	144.37
	3	31.46	85.79	142.99
	6	25.72	82.93	148.71
	9	28.59	88.60	160.51
	12	31.40	85.79	145.85

	SEd	CD
Cultures	1.95	3.85
Concentration	1.78	3.52
Days	1.38	2.72
Cultures x Concentration	4.37	8.62
Cultures x Concentration x Days	7.57	14.93

Fig 8. Effect of butachlor on chlorophyll content of the herbicide tolerant cyanobacterial isolates



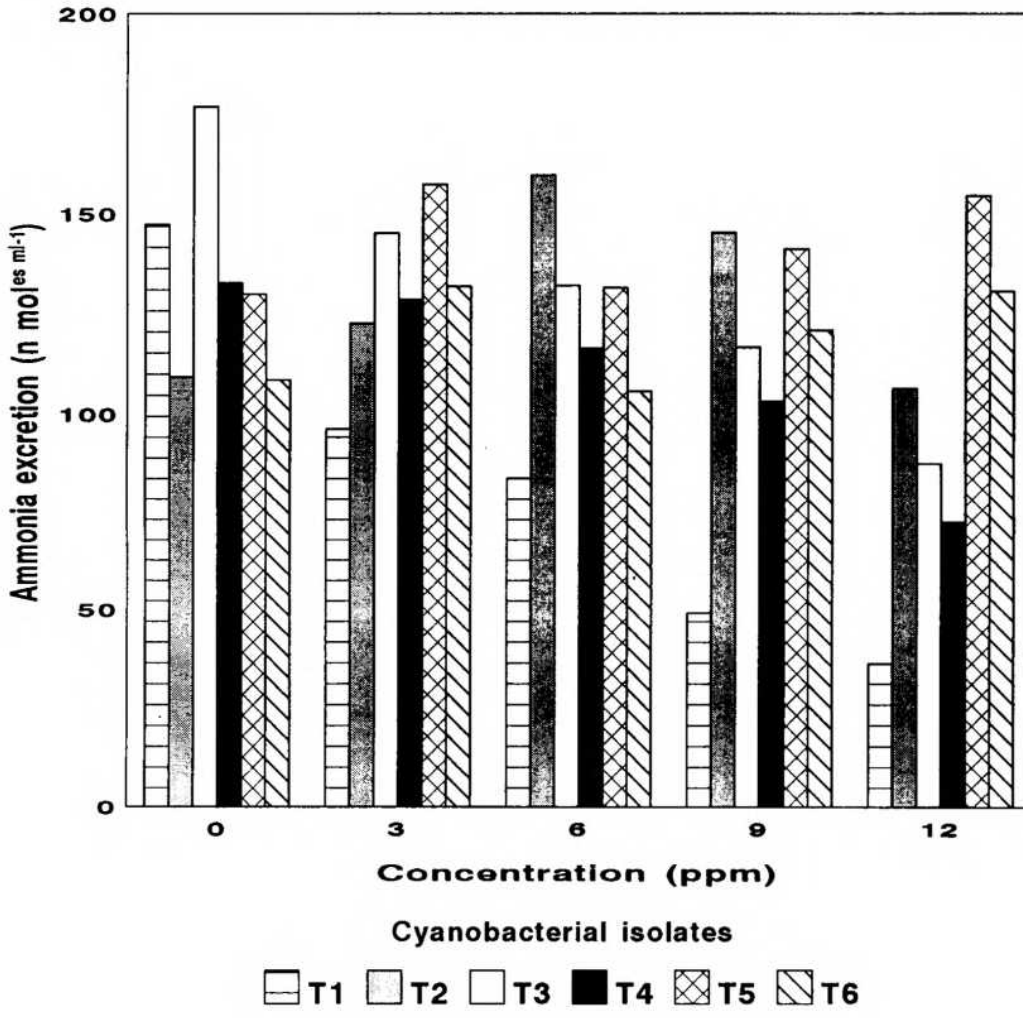
T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 13. Effect of butachlor on the ammonia excretion (n moles ml^{-1}) by the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	100.17	124.07	147.28
	3	85.31	102.27	96.18
	6	79.40	91.03	83.70
	9	52.64	52.12	49.07
	12	31.44	48.43	36.18
Anabaena-HT-SGK-2	0	96.15	136.15	109.27
	3	106.82	120.12	122.54
	6	138.96	164.26	159.73
	9	138.47	154.54	145.21
	12	121.43	116.32	106.31
Nostoc-HT-SGK-I	0	153.80	187.98	176.9
	3	128.12	156.60	145.07
	6	115.36	140.99	132.04
	9	102.53	125.25	116.69
	12	87.39	94.59	87.53
Oscillatoria-HT-SGK-I	0	115.36	140.99	132.63
	3	112.56	137.57	128.50
	6	107.53	125.25	116.39
	9	90.52	109.64	103.29
	12	63.25	80.08	72.50
Westiellopsis-HT-SGK-I	0	141.08	172.34	162.35
	3	179.92	211.35	196.71
	6	142.80	174.35	164.39
	9	172.76	185.00	176.37
	12	168.14	200.00	193.20
Westiellopsis-HT-SGK-2	0	118.58	144.93	135.64
	3	114.92	176.92	164.71
	6	115.24	140.85	132.13
	9	131.74	161.10	151.14
	12	140.44	171.31	163.29

	SEd	CD
Cultures	2.67	5.26
Concentration	2.43	4.80
Days	1.88	3.72
Cultures x Concentration	5.97	11.78
Cultures x Concentration x Days	10.34	20.40

Fig 9. Effect of butachlor on the ammonia excretion by the herbicide tolerant cyanobacterial isolates



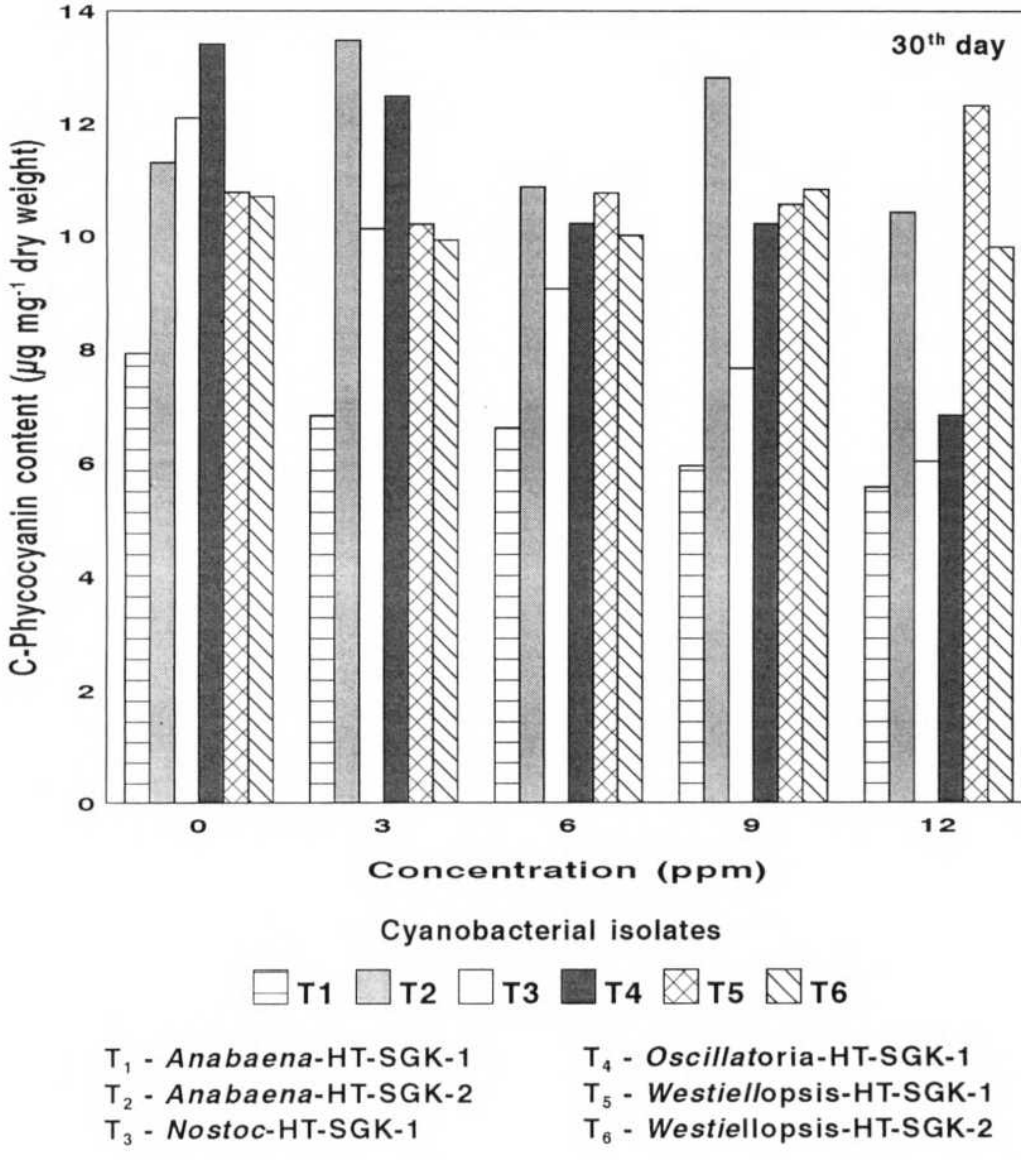
T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westiello*psis-HT-SGK-2

Table 14. Effect of butachlor on the C-Phycocyanin content (μg mg^{-1} dry weight)of the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	2.66	5.58	7.92
	3	1.75	4.84	6.82
	6	1.54	4.18	6.60
	9	0.88	3.74	5.94
	12	0.66	3.45	5.57
Anabaena-HT-SGK-2	0	1.95	7.16	11.29
	3	2.17	7.19	13.47
	6	2.82	7.82	10.86
	9	2.58	8.90	12.81
	12	1.92	6.30	10.42
Nostoc-HT-SGK-I	0	3.28	7.66	12.09
	3	2.73	6.56	10.12
	6	2.43	6.58	9.06
	9	2.18	4.92	7.66
	12	1.64	3.83	6.02
Oscillatoria-HT-SGK-I	0	2.35	5.74	13.40
	3	2.15	5.47	12.48
	6	2.06	5.56	10.21
	9	2.06	5.53	10.21
	12	1.36	3.01	6.84
Westiellopsis-HT-SGK-I	0	3.84	10.08	19.22
	3	4.54	11.18	18.22
	6	3.84	10.48	19.21
	9	3.99	12.23	18.85
	12	3.87	9.78	22.00
Westiellopsis-HT-SGK-2	0	3.01	10.99	19.09
	3	3.74	10.39	17.71
	6	3.01	9.90	17.87
	9	3.38	10.62	19.32
	12	3.79	10.20	17.51

	SEd	CD
Cultures	0.17	0.35
Concentration	0.16	0.32
Days	0.12	0.24
Cultures x Concentration	0.39	0.78
Cultures x Concentration x Days	0.69	1.36

Fig 10. Effect of butachlor on the C-phycocyanin content of the herbicide tolerant cyanobacterial isolates



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westiello*psis-HT-SGK-2

Anabaena-HT-SGK-1. Interestingly no reduction was observed in *Anabaena*-HT-SGK-2, *Westiellopsis*-HI-SGK-1 and *Westiellopsis*-HT-SGK-2 even at 12 ppm levels. Nearly 50 per cent inhibition of C-phycocyanin content was noticed on the 30th day in *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1 at 12 ppm of butachlor.

4.3.5. Effect of butachlor on the C-phycoerythrin content of the herbicide tolerant cyanobacterial isolates

The results are presented in Table 15. and Fig.11. No marked inhibition was seen at 12 ppm concentration in *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2. However the C-phycoerythrin content was significantly reduced to 50% in *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1 at all the observation intervals. Though *Anabaena* cultures recorded the minimum C-phycoerythrin content no marked inhibition was noticed due to butachlor at all concentrations.

4.3.6. Effect of butachlor on the allophycocyanin content of the herbicide tolerant cyanobacterial isolates

The results are presented in Table 16. and Fig.12.On the 10th day of observation butachlor was found to be inhibitory at all concentration in the case of *Anabaena*-HT-SGK-1, *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1. Interestingly *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 were found to tolerate butachlor concentrations upto 12 ppm while *Anabaena*-HT-SGK-1 and *Nostoc*-HT-SGK-1 were found to be inhibited even at 3ppm level. *Anabaena*-HT-SGK-2 could tolerate butachlor concentration upto 9 ppm without any significant reduction in the allophycocyanin content.

4.3.7. Effect of butachlor on the protein content of the herbicide tolerant cyanobacterial isolates

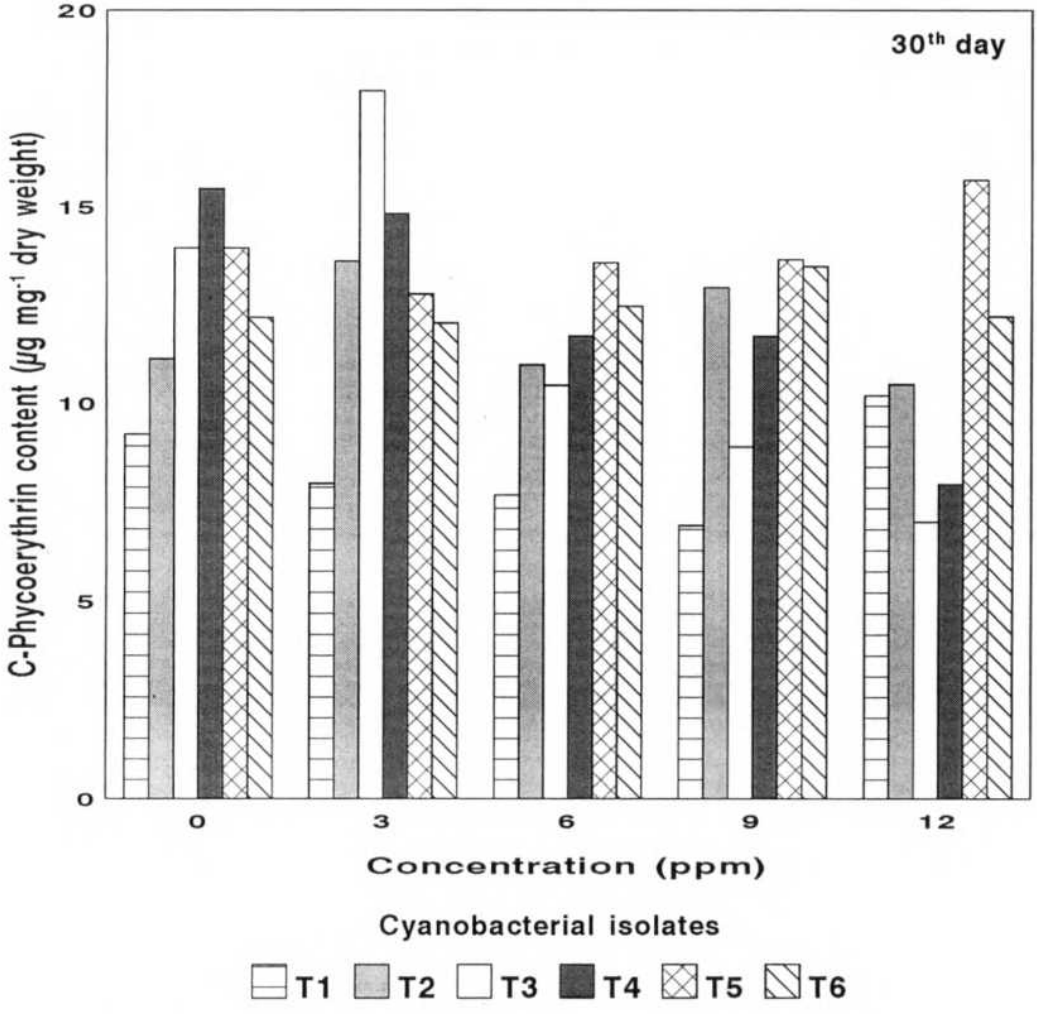
The results are presented in Table 17. and Fig.13. In general the protein content increased linearly during the incubation period. On the 10^{th} day of observation butachlor at all concentrations was found to be inhibitory to *Anabaena*-

Table 15. Effect of butachlor on C-Phycoerythrin content (µg mg^{-1} dryweight) of the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	2.64	5.50	9.19
	3	1.76	4.84	7.94
	6	1.54	4.18	7.65
	9	0.88	3.74	6.89
	12	0.66	3.45	10.21
Anabaena-HT-SGK-2	0	1.97	7.52	11.14
	3	2.19	7.14	13.63
	6	2.85	7.92	10.99
	9	2.81	9.01	12.97
	12	1.94	6.37	10.50
Nostoc-HT-SGK-I	0	3.80	8.88	13.96
	3	3.17	7.61	17.93
	6	2.85	6.66	10.47
	9	2.53	5.70	8.88
	12	1.90	4.44	6.98
Oscillatoria-HT-SGK-I	0	2.72	6.66	15.44
	3	2.50	6.34	14.83
	6	2.41	6.41	11.73
	9	2.21	6.36	11.73
	12	1.52	3.49	7.93
Westiellopsis-HT-SGK-I	0	3.49	9.15	17.45
	3	4.13	10.16	16.00
	6	3.39	8.48	17.00
	9	3.53	9.00	17.10
	12	3.51	8.07	19.60
Westiellopsis-HT-SGK-2	0	2.63	9.61	15.25
	3	3.31	8.97	15.08
	6	2.71	8.75	15.62
	9	3.08	9.35	16.88
	12	3.31	9.04	15.30

	SEd	CD
Cultures	1.96	3.88
Concentration	1.79	3.54
Days	1.39	2.74
Cultures x Concentration	4.40	8.68
Cultures x Concentration x Days	7.62	15.04

Fig 11. Effect of butachlor on the C-Phycoerythrin content of the herbicide tolerant cyanobacterial isolates



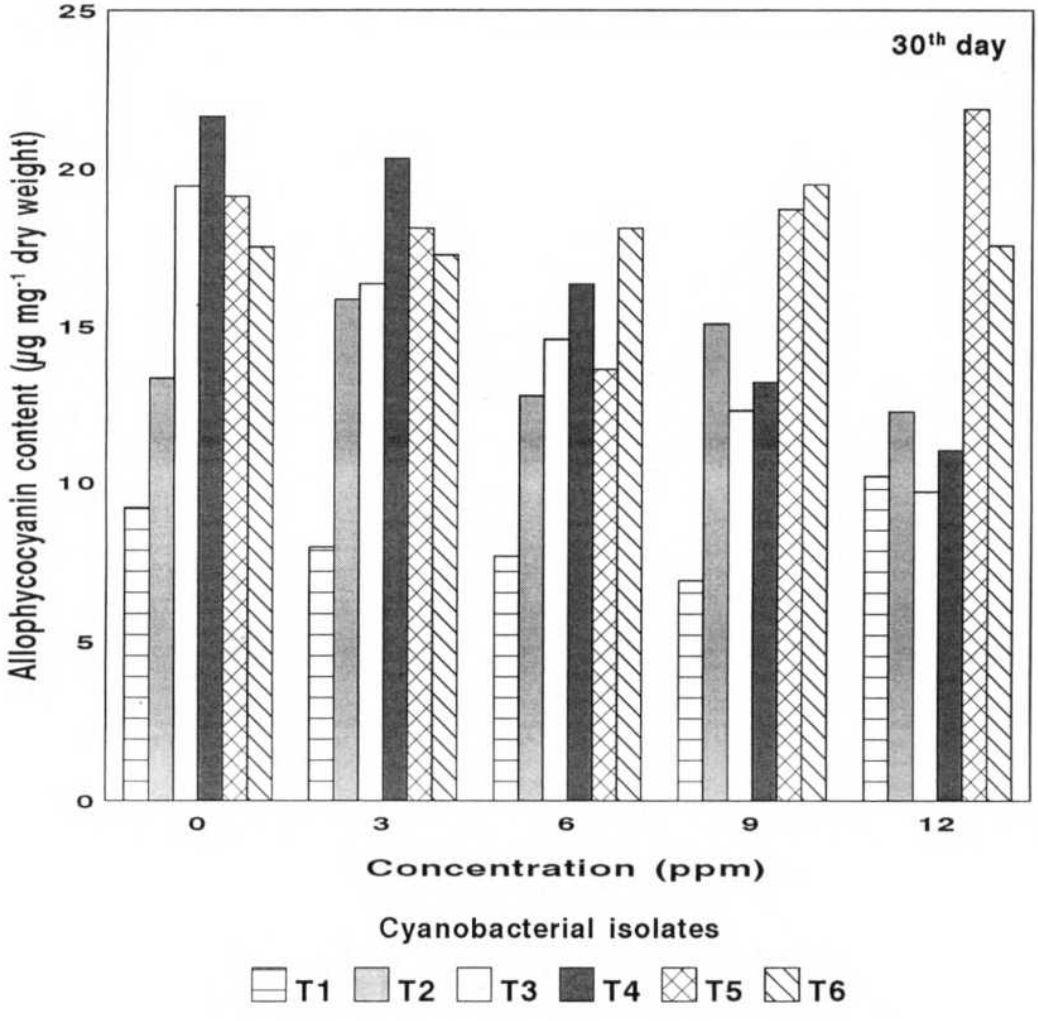
T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 16. Effect of butachlor on allophycocyanin content (μg mg^{-1} dryweight) of the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	3.06	6.41	9.19
	3	2.04	5.61	7.94
	6	1.78	4.48	7.65
	9	1.02	4.43	6.89
	12	0.70	4.07	10.21
Anabaena-HT-SGK-2	0	2.30	8.43	13.33
	3	2.55	8.30	15.85
	6	3.32	8.30	12.78
	9	3.04	10.94	15.08
	12	2.26	10.48	12.27
Nostoc-HT-SGK-I	0	5.30	12.37	19.44
	3	4.41	10.60	16.34
	6	3.97	9.27	14.58
	9	3.53	7.95	12.31
	12	3.00	7.18	9.72
Oscillatoria-HT-SGK-I	0	3.80	9.27	21.64
	3	3.78	8.83	20.32
	6	3.36	8.93	16.34
	9	3.08	8.86	13.22
	12	2.20	4.86	11.04
Westiellopsis-HT-SGK-I	0	6.02	16.04	30.58
	3	7.19	17.69	28.97
	6	6.05	16.60	21.80
	9	6.22	19.40	29.94
	12	6.02	15.50	35.00
Westiellopsis-HT-SGK-2	0	4.80	17.69	28.00
	3	6.10	16.52	27.60
	6	4.86	15.94	28.96
	9	5.44	17.11	31.20
	12	6.02	16.52	28.10

	SEd	CD
Cultures	0.42	0.83
Concentration	0.38	0.76
Days	0.30	0.59
Cultures x Concentration	0.67	1.32
Cultures x Concentration x Days	1.64	3.25

Fig 12. Effect of butachlor on the Allophycocyanin content of the herbicide tolerant cyanobacterial isolates



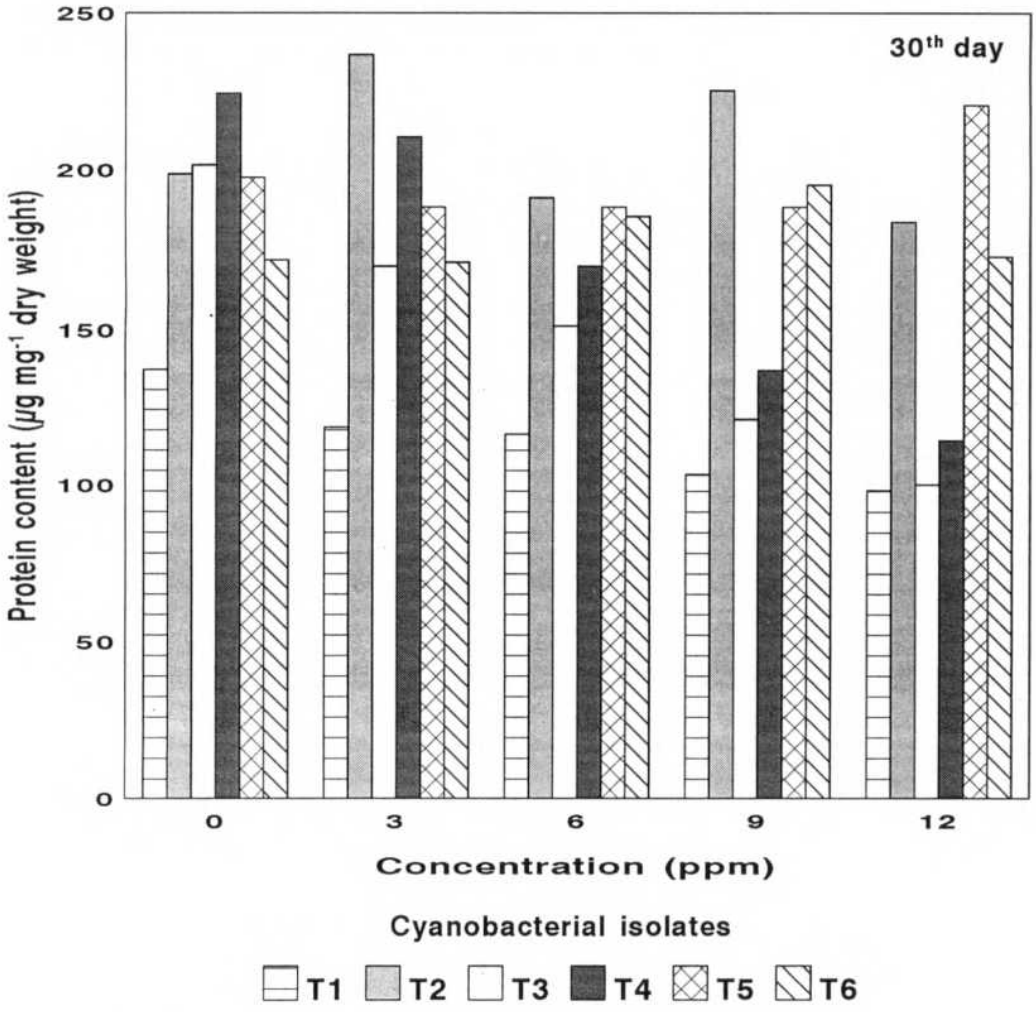
T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2

Table 17. Effect of butachlor on protein content (µg mg^{-1} dry weight) of the herbicide tolerant cyanobacterial isolates

Isolates	Butachlor conc. (ppm)	Days		
		10	20	30
Anabaena-HT-SGK-1	0	45.71	95.23	137.31
	3	30.47	85.80	118.58
	6	26.60	72.38	116.36
	9	15.23	64.76	103.28
	12	11.23	60.75	98.32
Anabaena-HT-SGK-2	0	34.35	126.02	198.58
	3	38.10	137.49	236.78
	6	49.63	150.57	190.95
	9	45.45	156.30	225.32
	12	33.70	110.97	183.32
Nostoc-HT-SGK-I	0	54.93	121.18	201.44
	3	45.70	109.87	169.39
	6	41.20	96.14	151.08
	9	36.62	82.40	121.18
	12	27.64	64.09	100.12
Oscillatoria-HT-SGK-I	0	41.20	96.14	224.33
	3	40.20	91.56	210.50
	6	36.62	91.04	169.39
	9	32.04	92.16	137.14
	12	22.59	50.36	114.36
Westiellopsis-HT-SGK-I	0	50.36	150.88	276.39
	3	61.76	149.76	263.20
	6	51.00	141.77	263.22
	9	61.17	163.07	263.20
	12	60.05	131.70	308.90
Westiellopsis-HT-SGK-2	0	42.35	141.17	240.00
	3	51.76	139.67	237.00
	6	41.16	136.47	259.12
	9	47.05	145.88	273.12
	12	50.06	140.17	241.35

	SEd	CD
Cultures	2.51	4.96
Concentration	2.29	4.52
Days	1.77	3.50
Cultures x Concentration	5.62	11.09
Cultures x Concentration x Days	9.73	19.21

Fig 13. Effect of butachlor on the protein content of the herbicide tolerant cyanobacterial isolates



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westiello*psis-HT-SGK-2

HT-SGK-1. *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 were found to be tolerant by registering the maximum protein content even at 12ppm of butachlor. In the case of the isolate *Anabaena*-HT-SGK-2 the protein content was not found to be affected by 9 ppm of butachlor.

4.3.8. Effect of butachlor on the nitrogenase activity of the herbicide tolerant cyanobacterial isolates

The results are presented in Table 18. and Fig.14. In general, butachlor at all concentrations was found to be inhibitory to the nitrogenase activity of *Anabaena*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1, and *Oscillatoria*-HT-SGK-1. In the absence of butachlor *Westiellopsis* HT-SGK-2 showed maximum nitrogenase activity followed by *Westiellopsis*-HT-SGK-1.

4.4. MOLECULAR CHARACTERIZATION OF THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES

The results are presented in Plate 14 . When agarose gel electrophoresis was performed with the total DNA extracted from the herbicide tolerant cyanobacterial isolates, the appearance of a single intact band corresponding to the 23 kb position of the marker denotes the chromosomal DNA. The absence of any bands in the lower portions of the gel reveals that the low molecular weight plasmids are not present in any of the herbicide tolerant cyanobacterial isolates.

4.5. EFFECT OF INOCULATION OF HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES ON ADT 36 RICE SEEDLINGS

4.5.1. Effect of inoculation of herbicide tolerant cyanobacterial isolates on plant height of ADT 36 rice seedlings

The results are presented in Table 19, Fig.15. Plate 15 shows the biomass establishment by the herbicide tolerant cyanobacterial isolates in soils planted with ADT 36 rice seedlings. In general individual inoculation of the cyanobacterial isolates with butachlor increased plant height markedly over uninoculated control.

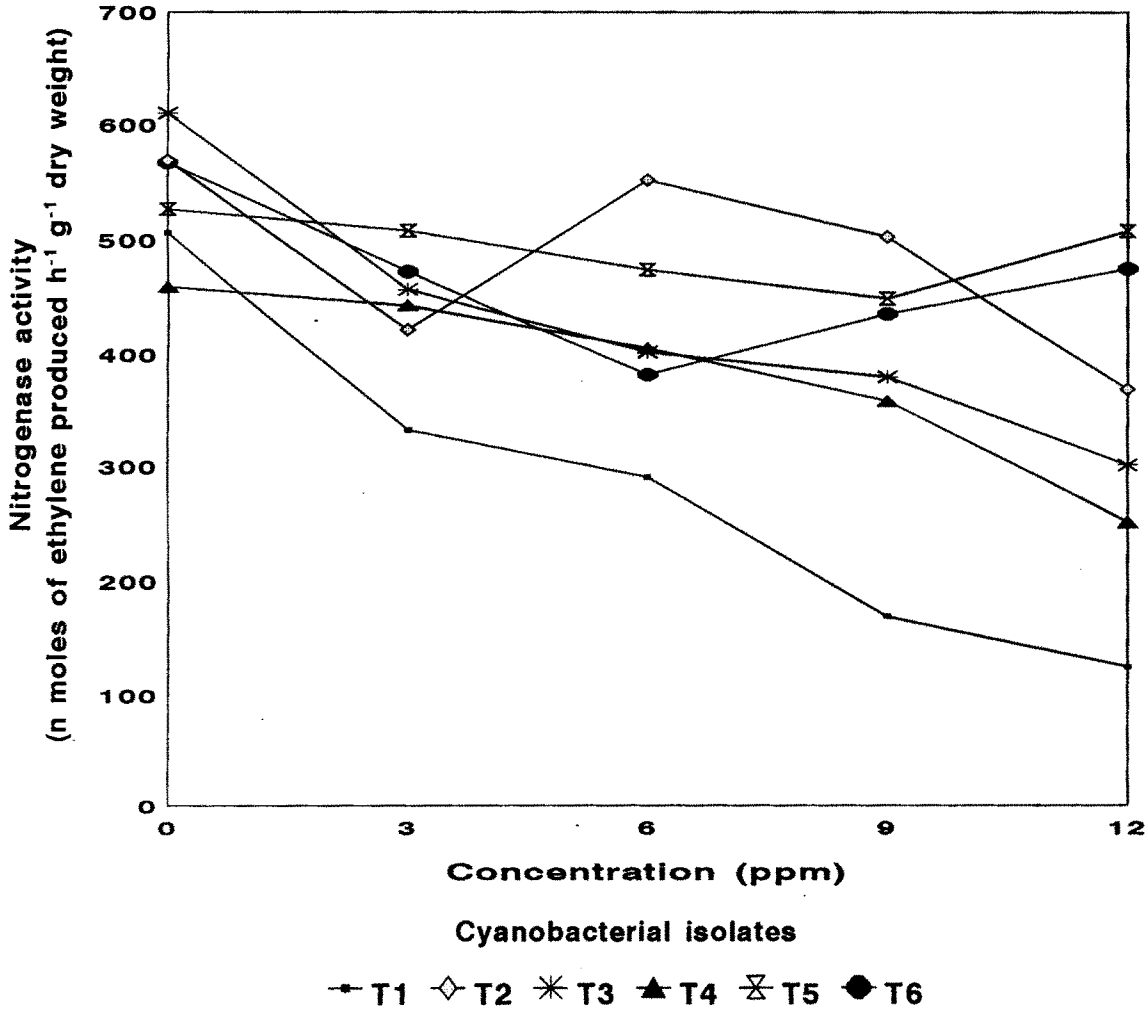
Table 18. Effect of butachlor on the nitrogenase activity * of the herbicide tolerant cyanobacterial isolates

Cyanobacterial isolates	Butachlor conc. (ppm)	Nitrogenase activity
Anabaena-HT-SGK-1	0	506.08
	3	332.27
	6	290.08
	9	169.82
	12	125.39
Anabaena-HT-SGK-2	0	568.68
	3	422.00
	6	551.73
	9	503.22
	12	368.40
Nostoc-HT-SGK-I	0	609.97
	3	457.47
	6	402.00
	9	379.64
	12	301.15
Oscillatoria-HT-SGK-I	0	459.66
	3	443.51
	6	405.51
	9	357.97
	12	251.26
Westiellopsis-HT-SGK-I	0	902.51
	3	870.71
	6	813.83
	9	771.00
	12	871.30
Westiellopsis-HT-SGK-2	0	971.64
	3	811.00
	6	654.12
	9	747.54
	12	816.00

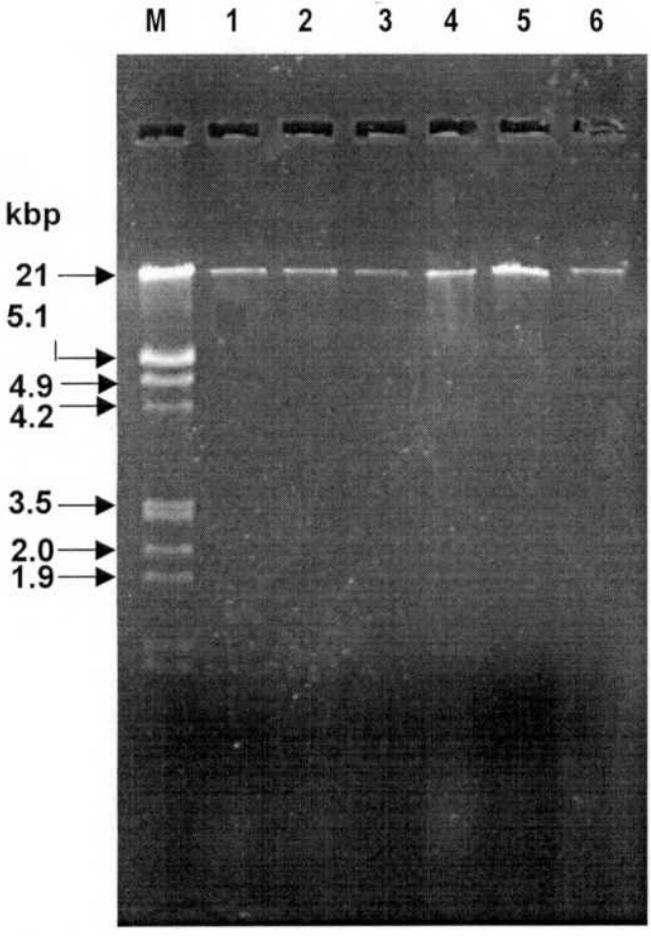
* n moles of ethylene produced $h^{-1}g^{-1}$ dry weight

	SEd	CD
Cultures	15.68	31.36
Concentration	14.31	28.63
Cultures x Concentration	35.06	70.14

Fig 14. Effect of butachlor on the nitrogenase activity of the herbicide tolerant cyanobacterial isolates



T_1 - *Anabaena*-HT-SGK-1
T_2 - *Anabaena*-HT-SGK-2
T_3 - *Nostoc*-HT-SGK-1
T_4 - *Oscillato*ria-HT-SGK-1
T_5 - *Westiello*psis-HT-SGK-1
T_6 - *Westie*llopsis-HT-SGK-2



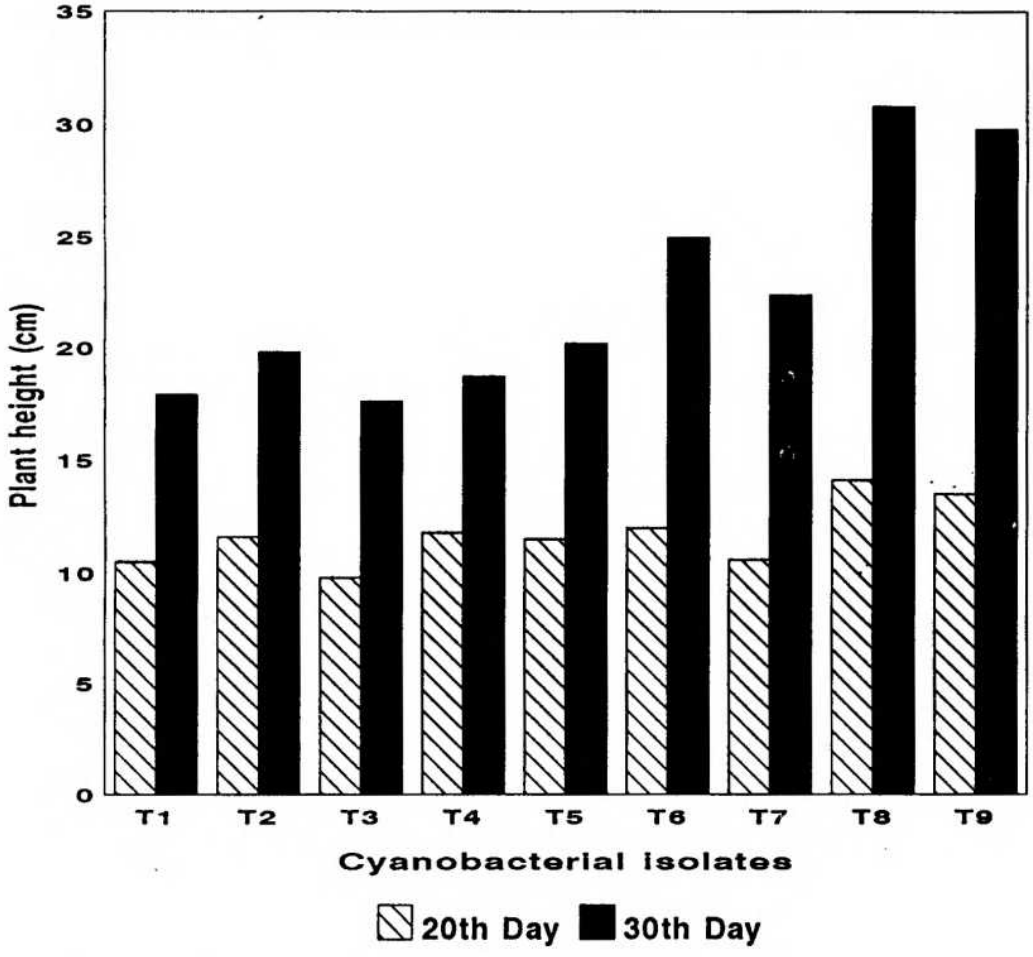
M - Marker
1 – *Westiellopsis*-HT-SGK-1
2 - *Westiellopsis*-HT-SGK-2
3 – *Anabaena*-HT-SGK-1
4 - *Anabaena*-HT-SGK-2
5 – *Nostoc*-HT-SGK-1
6 –*Oscillatoria*-HT-SGK-1

Plate 14. Total DNA extract of the herbicide tolerant cyanobacterial isolates

Table 19. Effect of inoculation of herbicide tolerant cyanobacterial isolates on plant height of ADT 36 rice seedlings

Treatment	Plant height (cm)	
	20th day	30th day
T_1 - Control	10.5	17.9
T_2 - *Anabaena*-HT-SGK-1 + butachlor 30µl	11.6	19.8
T_3 - *Anabaena* -HT-SGK-2 + butachlor 30µl	9.8	17.6
T_4 - *Nostoc*-HT-SGK-1 + butachlor 30µl	11.8	18.7
T_5 - *Oscillatoria*-HT-SGK-1 + butachlor 30µl	11.5	20.2
T_6 - *Westiellopsis*-HT-SGK-1 + butachlor 30µl	12.0	25.0
T_7 - *Westiellopsis*-HT-SGK-2 + butachlor 30µl	10.6	22.4
T_8 - Composite inoculum + butachlor 30µl	14.1	30.8
T_9 - Composite inoculum - butachlor 30µl	13.5	29.8
	SEd	CD
Days	0.74	1.52
Treatment	1.58	3.22
Days x Treatment	2.24	4.56

Fig 15. Effect of inoculation of herbicide tolerant cyanobacterial isolates on plant height of ADT 36 rice seedlings



T_1 - Control
T_2 - *Anabaena*-HT-SGK-1
T_3 - *Anabaena*-HT-SGK-2
T_4 - *Nostoc*-HT-SGK-1
T_5 - *Oscillatoria*-HT-SGK-1
T_6 - *Westiellopsis*-HT-SGK-1+Butachlor
T_7 - *Westiellopsis*-HT-SGK-2+Butachlor
T8 - Composite cyanobacterial isolates+butachlor
T9 - Composite cyanobacterial isolates-butachlor

Plate 15 **Biomass establishment by the herbicide tolerant cyanobacterial isolates in soils planted with ADT 36 rice seedlings**

Significant increase in plant height was noticed with the inoculation of composite cultures both in the presence and absence of butachlor. The results clearly indicated that the herbicide tolerant isolates performed better in improving the plant height of ADT 36 rice seedlings.

4.5.2. Effect of inoculation of the herbicide tolerant cyanobacterial isolates on the total chlorophyll content of ADT 36 rice seedlings

The results are presented in Table 20 and Fig.16. Significant increase in the total chlorophyll content was observed due to inoculation of the herbicide tolerant cyanobacterial isolates. However the maximum chlorophyll content was recorded with the inoculation of composite cultures in the presence of butachlor at both intervals.

4.5.3. Effect of inoculation of the herbicide tolerant cyanobacterial isolates on the ammonia excretion pattern in flood water in soils planted with ADT 36 rice seedlings

The results are presented in Table 21 and Fig.17. The ammonia excretion increased linearly due to inoculation. Maximum ammonia excretion was recorded with inoculation of the composite cultures both in the presence and absence of butachlor. Among the individual treatments *Westiellopsis*-HT-SGK-2 showed maximum levels of ammonia, while *Anabaena*-HT-SGK-2 showed the minimum ammonia excretion in flood water.

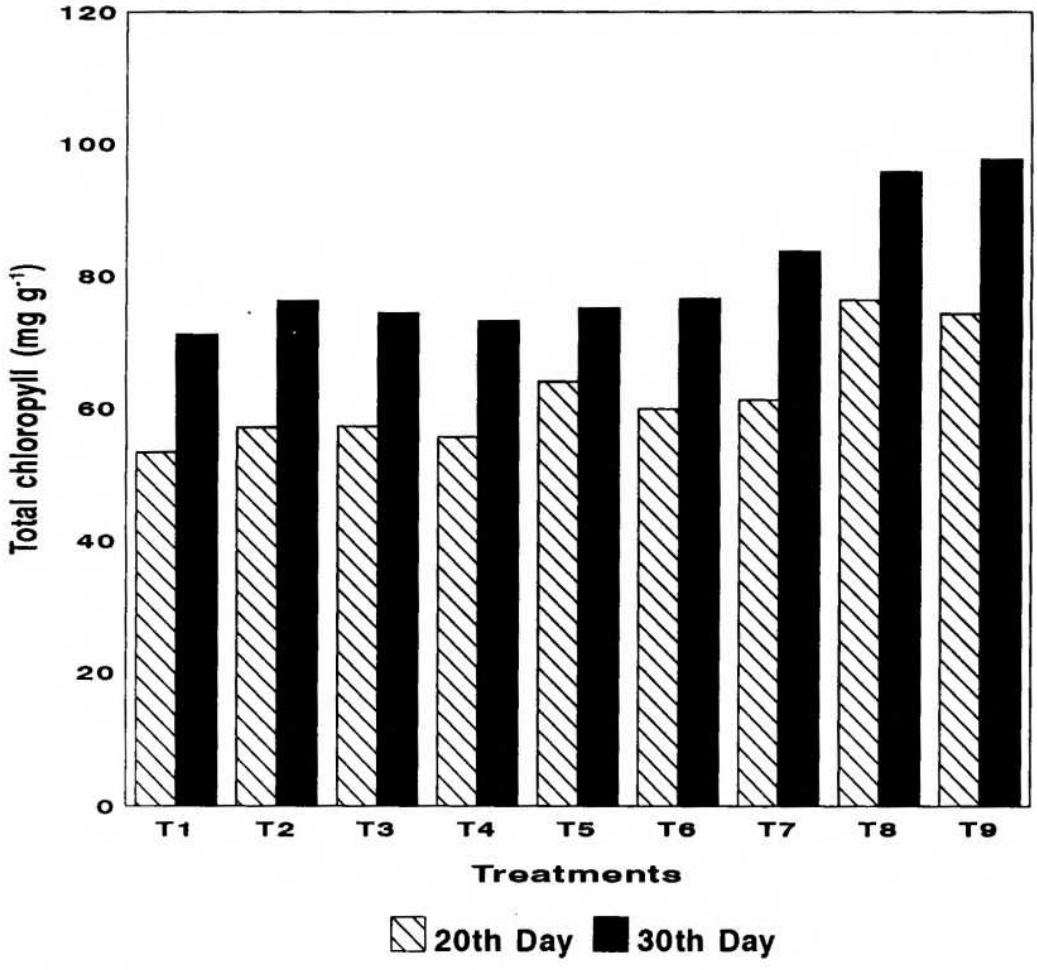
4.5.4. Effect of inoculation of the herbicide tolerant cyanobacterial isolates on the total nitrogen content of ADT 36 rice seedlings

The results are presented in Table 22 and Fig.18. Significant increase was noticed due to the inoculation of the herbicide tolerant cyanobacterial isolates when applied individually or as composite culture both in the presence and absence of butachlor. Among the individual treatments, *Westiellopsis*-HT-SGK-2 recorded the

Table 20. Effect of inoculation of herbicide tolerant cyanobacterial isolates on total chlorophyll of ADT 36 rice seedlings

Treatment	Total chlorophyll (mg g^{-1} fresh weight)	
	20th Day	30th Day
T_1 .Control	53.31	71.00
T_2 - *Anabaena*-HT-SGK-1 + butachlor 30µl	57.10	76.10
T_3 - *Anabaena* -HT-SGK-2 + butachlor 30µl	57.23	74.30
T_4. *Nostoc*-HT-SGK-1 + butachlor 30µl	55.67	73.09
T_5 - *Oscillatoria*-HT-SGK-1 + butachlor 30µl	63.99	75.00
T_6 - *Westiellopsis*-HT-SGK-1 + butachlor 30µl	59.90	76.50
T_7 - *Westiellopsis*-HT-SGK-2 + butachlor 30µl	61.26	83.70
T_8. Composite inoculum + butachlor 30µl	76.33	97.60
T_9 - Composite inoculum - butachlor 30µl	74.21	95.70
	SEd	CD
Days	0.83	1.69
Treatment	1.76	3.59
Days x Treatment	2.50	5.08

Fig 16. Effect of inoculation of herbicide tolerant cyanobacterial isolates on total chlorophyll of ADT 36 rice seedlings

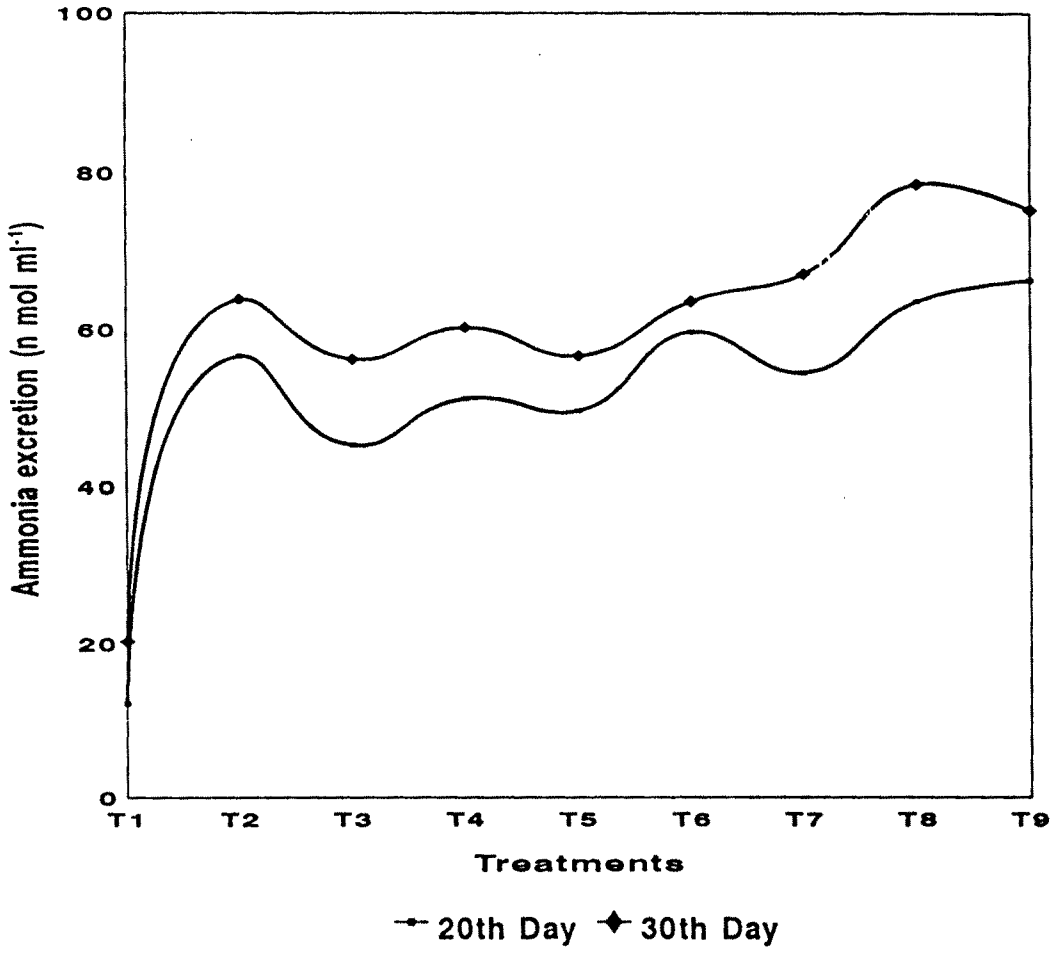


T_1 - Control
T_2 - *Anabaena*-HT-SGK-1
T_3 - *Anabaena*-HT-SGK-2
T_4 - *Nostoc*-HT-SGK-1
T_5 - *Oscillatoria*-HT-SGK-1
T_6 - *Westiellopsis*-HT-SGK-1 + Butachlor
T_7 - *Westiellopsis*-HT-SGK-2 + Butachlor
T8 - Composite cyanobacterial isolates + butachlor
T9 - Composite cyanobacterial isolates-butachlor

Table21. Effect of inoculation of herbicide tolerant cyanobacterial isolates on ammonia excretion in flood water of soils planted with ADT-36 rice seedlings

Treatment	Ammonia excretion (n mol ml^{-1})	
	20^{th} Day	30^{th} Day
T_1 .Control	12.23	20.20
T_2 - *Anabaena*-HT-SGK-1 + butachlor 30µl	56.50	63.76
T_3 - *Anabaena* -HT-SGK-2 + butachlor 30µl	45.20	56.10
T_4. *Nostoc*-HT-SGK-1 + butachlor 30µl	51.06	60.15
T_5 - *Oscillatoria*-HT-SGK-1 + butachlor 30µl	49.50	56.50
T_6 - *Westiellopsis*-HT-SGK-1 + butachlor 30µl	59.60	63.50
T_7 - *Westiellopsis*-HT-SGK-2 + butachlor 30µl	54.37	67.00
T_8 . Composite inoculum + butachlor 30µl	66.16	78.50
T_9 - Composite inoculum - butachlor 30µl	63.50	75.16
	SEd	CD
Days	3.10	6.29
Treatment	1.46	2.96
Days x Treatment	4.38	8.90

Fig 17. Effect of inoculation of herbicide tolerant cyanobacterial isolates on ammonia excretion in flood water of soils planted with ADT 36 rice seedlings

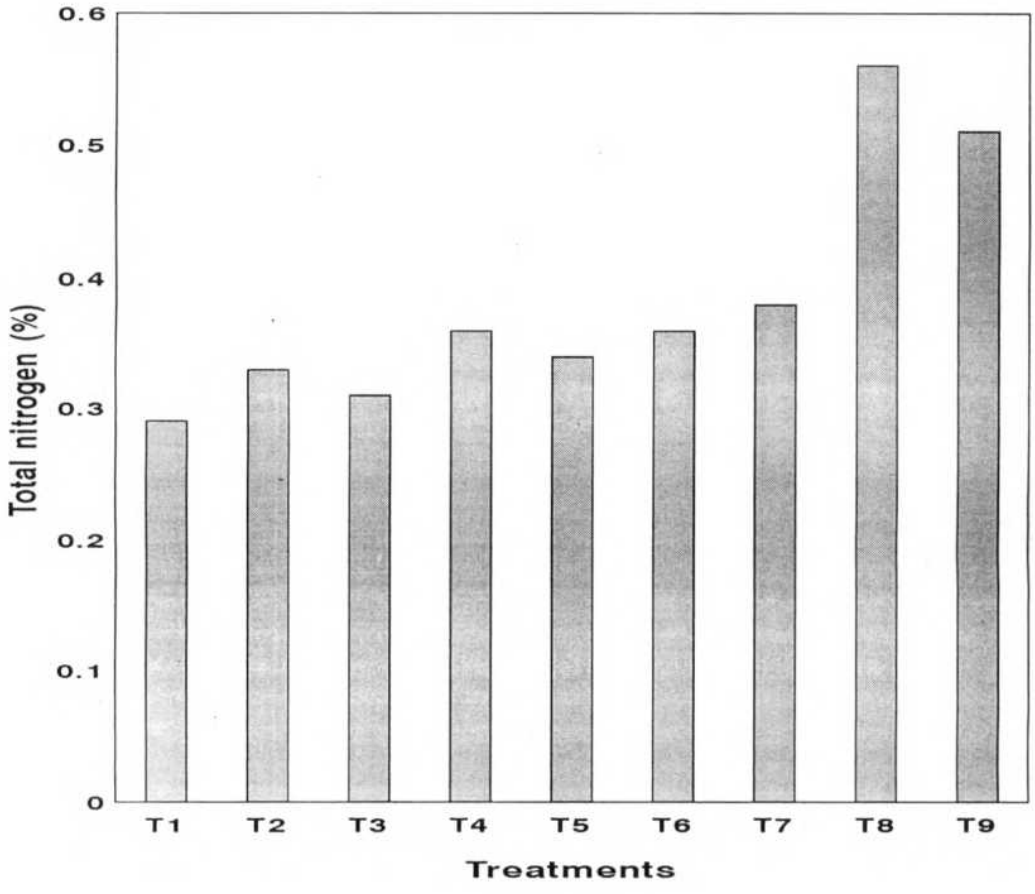


T_1 - Control
T_2 - *Anabaena*-HT-SGK-1
T_3 - *Anabaena*-HT-SGK-2
T_4 - *Nostoc*-HT-SGK-1
T_5 - *Oscillatoria*-HT-SGK-1
T_6 - *Westiellopsis*-HT-SGK-1 + Butachlor
T_7 - *Westiellopsis*-HT-SGK-2 + Butachlor
T8 - Composite cyanobacterial isolates + butachlor
T9 - Composite cyanobacterial isolates-butachlor

Table 22. Effect of inoculation of herbicide tolerant cyanobacterial isolates on the total nitrogen content of ADT-36 rice seedlings

Treatment	Total Nitrogen %
T_1 .Control	0.29
T_2 - *Anabaena*-HT-SGK-1 + butachlor 30µl	0.33
T_3 - *Anabaena* -HT-SGK-2 + butachlor 30µl	0.31
T_4 . *Nostoc*-HT-SGK-1 + butachlor 30µl	0.36
T_5 - *Oscillatoria*-HT-SGK-1 + butachlor 30µl	0.34
T_6 - *Westiellopsis*-HT-SGK-1 + butachlor 30µl	0.36
T_7 - *Westiellopsis*-HT-SGK-2 + butachlor 30µl	0.38
T_8 . Composite inoculum + butachlor 30µl	0.56
T_9 - Composite inoculum - butachlor 30µl	0.51
SEd	0.01
CD	0.04

Fig 18. Effect of inoculation of herbicide tolerant cyanobacterial isolates on total nitrogen content of ADT 36 rice seedlings



T_1 - Control
T_2 - *Anabaena*-HT-SGK-1
T_3 - *Anabaena*-HT-SGK-2
T_4 - *Nostoc*-HT-SGK-1
T_5 - *Oscillatoria*-HT-SGK-1
T_6 - *Westiellopsis*-HT-SGK-1 + Butachlor
T_7 - *Westiellopsis*-HT-SGK-2 + Butachlor
T8 - Composite cyanobacterial isolates + butachlor
T9 - Composite cyanobacterial isolates-butachlor

maximum total N content of rice seedlings while *Anabaena*-HT-SGK-2 inoculation recorded the lowest total N content.

4.6. EFFECT OF INOCULATION OF THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES ALONG WITH GRADED LEVELS OF N ON THE YIELD OF ADT 36 RICE

4.6.1. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the plant biometrics of ADT 36 rice

The results are presented in Table 23 and Fig. 19 and Plates 16 to 19. Significant increase in plant height, total tillers and productive tillers was observed due to cyanobacterial inoculation. Inoculation of the herbicide tolerant cyanobacterial isolates along with 75% N increased the plant height on par with 100% N application. Interestingly, total tiller production was highest under 100% application but productive tillers were highest when cyanobacterial inoculation was done with 75% N.

4.6.2. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the yield attributes of ADT 36 rice

The results are presented in Table 24 and Fig. 20. The yield determining factors *viz.*, panicle length, number of filled grains per panicle and 1000 grain weight were significantly improved by cyanobacterial inoculation in the presence of N. However no marked variation could be seen due to inoculation in the absence of N application. Significant increase in the plant height, number of filled grains and 1000 grain weight was recorded only when inoculation was done along with 75% which was on par with 100% N application.

4.6.3. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the grain and straw yield of ADT 36 rice.

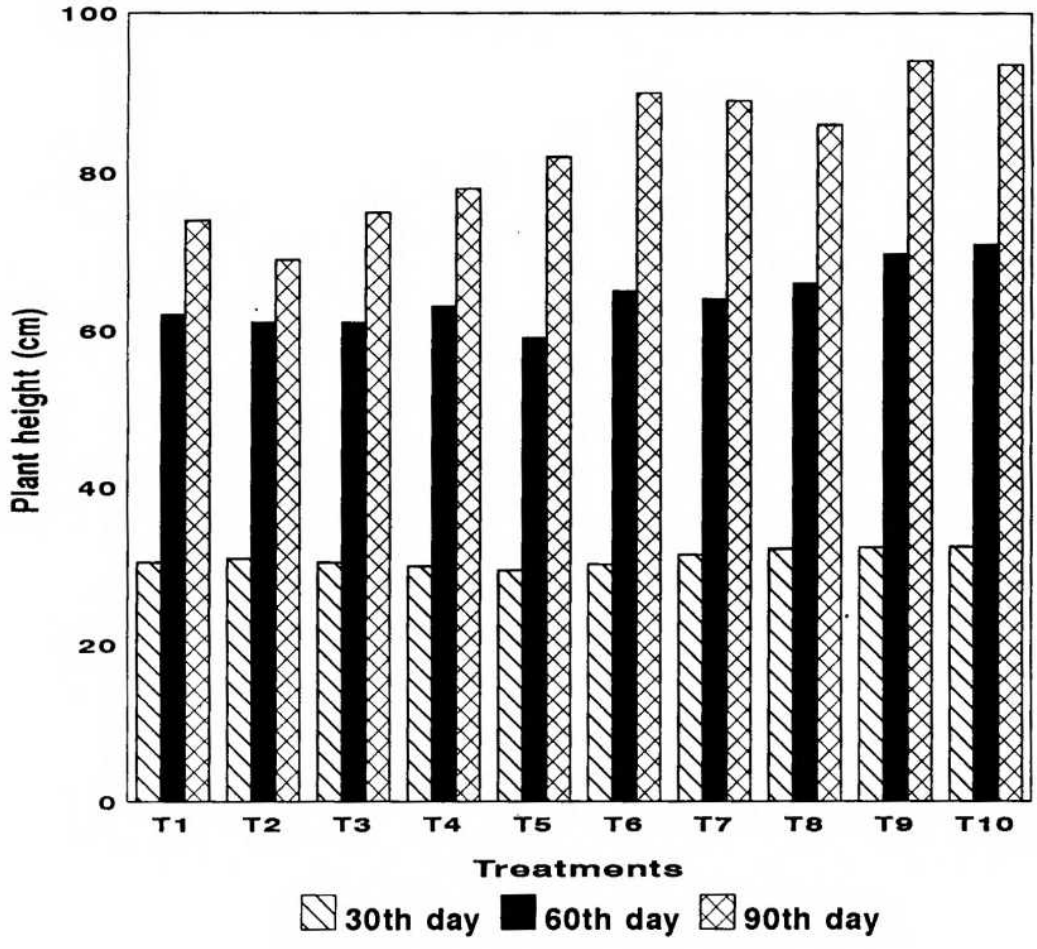
The results are presented in Table 25 and Fig. 21. The grain and straw yield of ADT 36 rice were significantly improved due to cyanobacterial inoculation. The application of 75% N along with inoculation of the herbicide tolerant cyanobacterial

Table 23. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on plant biometrics of ADT 36 rice

Treatment	Plant height (cm)			Total tillers hill^{-1}		Productive tillers hill^{-1}
	Days after transplanting					
	30	60	90	30	60	90
T_1 - H_0 I_0 N_0	30.5	62.0	74.0	4.6	5.3	3.6
T_2 - H_0 I_1 N_0	31.0	61.0	69.0	5.1	6.0	3.3
T_3 – H_1 I_0 N_0	30.5	61.0	75.0	4.6	5.0	3.0
T_4 – H_1 I_1 N_0	30.0	63.0	78.0	6.0	7.0	4.0
T_5 - H_0 I_0 (75% N)	29.5	59.0	82.0	6.3	8.0	6.0
T_6 - H_0 I_1 (75% N)	30.3	65.0	90.0	8.0	10.0	6.3
T_7 - H_1 I_0 (75% N)	31.5	64.0	89.0	6.3	7.2	4.3
T_8 – H_1 I_1 (75% N)	32.3	66.0	86.0	8.0	10.8	7.0
T_9 – H_1 I_0 (100% N)	32.5	69.8	94.0	9.6	12.6	7.9
T_{10} – H_1 I_1 (100% N)	32.6	71.0	93.5	9.0	11.1	7.6

	SEd	CD	SEd	CD	SEd – 0.35
Treatment	1.48	2.99	0.12	0.25	
Days	0.66	1.33	0.28	0.58	CD – 0.73
Treatment x days	2.09	4.23	0.40	0.82	

Fig 19. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the plant height of ADT 36 rice



T_1 - H_0 I_0 N_0
T_2 - H_0 I_1 N_0
T_3 - H_1 I_0 N_0
T_4 - H_1 I_1 N_0
T_5 - H_0 I_0 N75
T_6 - H_0 I_1 N75
T_7 - H_1 I_0 N75
T_8 - H_1 I_1 N75
T_9 - H_1 I_0 N100
T_10 - H_1 I_1 N100

Plate 16 **Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with 75% N on ADT 36 rice at flowering**

Plate 17 **Effect of application of 100% N on ADT 36 rice at flowering**

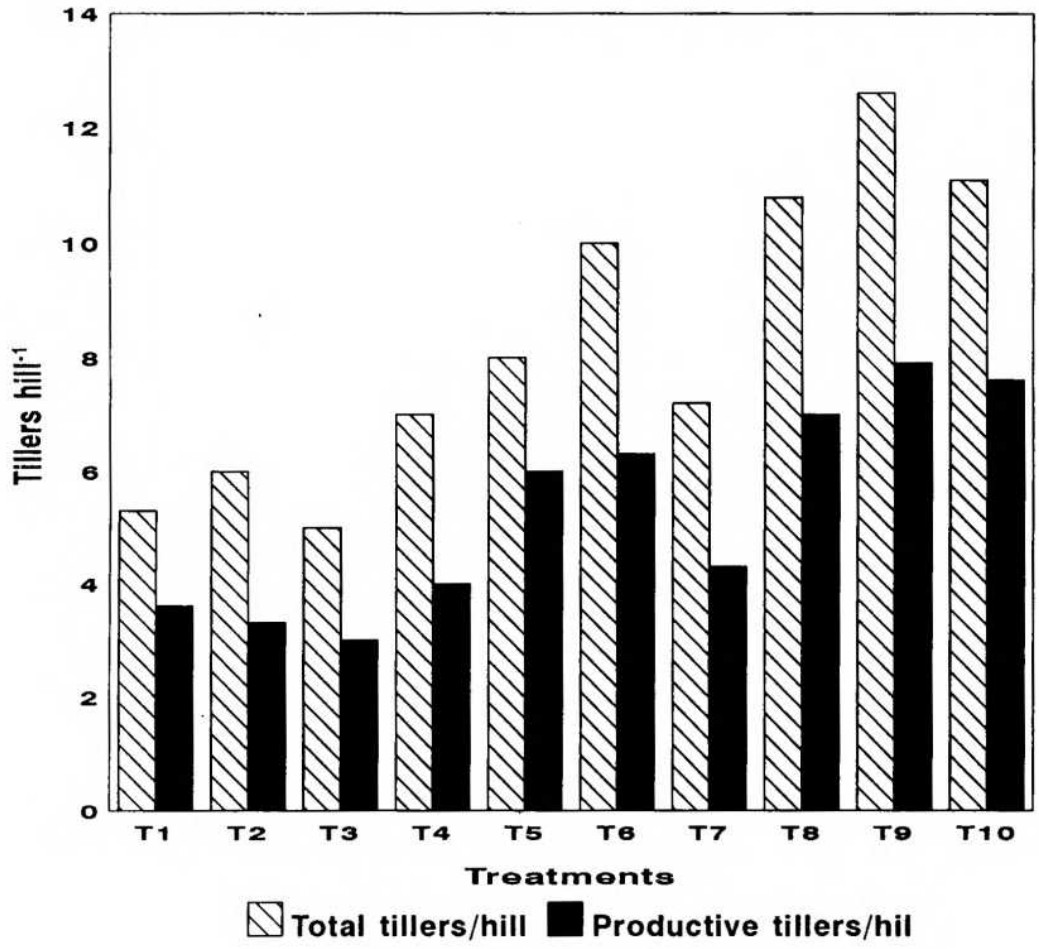
Plate 18 **Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with 75% N on ADT 36 rice at harvest**

Plate 19 **Effect of application of 100% N on ADT 36 rice at harvest**

Table 24. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the yield attributes of ADT 36 rice

Treatment	Panicle length (cm)	Number of filled grains panicle^{-1}	1000 grain weight(g)
T_1 - H_0 I_0 N_0	18.0	46.33	20.58
T_2 - H_0 I_1 N_0	20.33	45.33	22.50
T_3 – H_1 I_0 N_0	21.66	62.33	23.52
T_4 – H_1 I_1 N_0	20.00	62.00	23.52
T_5 - H_0 I_0 (75% N)	22.66	62.34	25.40
T_6 - H_0 I_1 (75% N)	24.00	81.00	26.15
T_7 - H_1 I_0 (75% N)	22.00	77.35	25.50
T_8 – H_1 I_1 (75% N)	25.00	102.26	26.90
T_9 – H_1 I_0 (100% N)	23.66	82.33	26.50
T_{10} – H_1 I_1 (100% N)	25.33	96.37	26.60
SEd	1.00	4.37	1.05
CD	2.08	9.11	2.19

Fig 20. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on total and productive tillers of ADT 36 rice

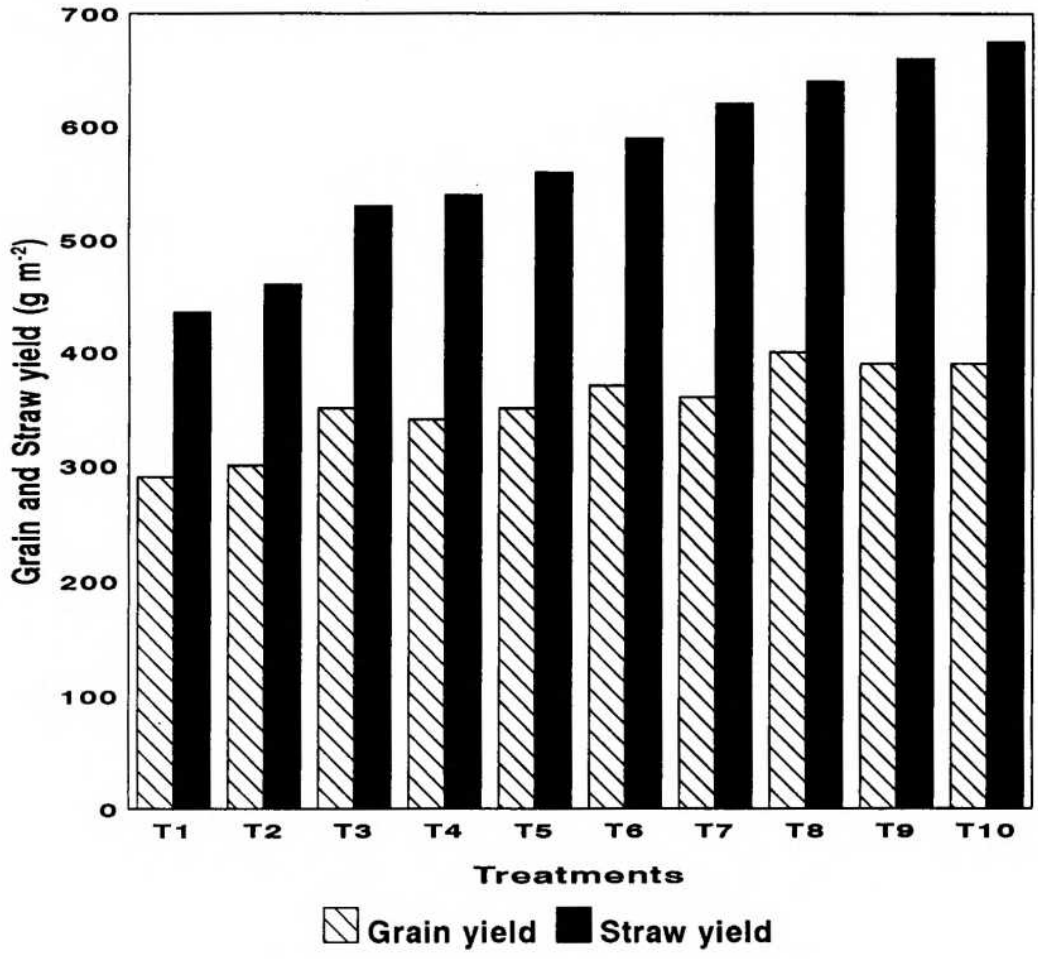


T_1 - H_0 I_0 N_0
T_2 - H_0 I_1 N_0
T_3 - H_1 I_0 N_0
T_4 - H_1 I_1 N_0
T_5 - H_0 I_0 N75
T_6 - H_0 I_1 N75
T_7 - H_1 I_0 N75
T_8 - H_1 I_1 N75
T_9 - H_1 I_0 N100
T_10 - H_1 I_1 N100

Table 25. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the yield of ADT 36 rice

Treatment	Grain Yield $(g\ m^{-2})^{-1}$	% Increase over control	Straw yield $(g\ m^{-2})^{-1}$	% increase over control
T_1 - H_0 I_0 N_0	290	-	435	-
T_2 - H_0 I_1 N_0	300	3.44	460	5.74
T_3 – H_1 I_0 N_0	350	20.68	530	21.80
T_4 – H_1 I_1 N_0	340	17.27	540	24.1
T_5 - H_0 I_0 (75% N)	350	20.68	560	28.7
T_6 - H_0 I_1 (75% N)	370	27.50	590	35.6
T_7 - H_1 I_0 (75% N)	360	34.48	620	42.5
T_8 – H_1 I_1 (75% N)	400	37.93	640	47.1
T_9 – H_1 I_0 (100% N)	390	34.48	660	51.7
T_{10} – H_1 I_1 (100% N)	390	34.48	675	55.17
SEd	15.40		26.90	
CD	32.12		56.11	

Fig 21. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on grain yield and straw yield of ADT 36 rice



T_1 - H_0 I_0 N_0
T_2 - H_0 I_1 N_0
T_3 - H_1 I_0 N_0
T_4 - H_1 I_1 N_0
T_5 - H_0 I_0 N75
T_6 - H_0 I_1 N75
T_7 - H_1 I_0 N75
T_8 - H_1 I_1 N75
T_9 - H_1 I_0 N100
T_10 - H_1 I_1 N100

isolates recorded the highest grain yield, which was higher than 100% N application. While the straw yield due to the combined inoculation of 75% N along with cyanobacterial inoculation was on par with 100% N application. The results clearly pointed out the positive influence of cyanobacterial inoculation on the yield of ADT 36 rice crop.

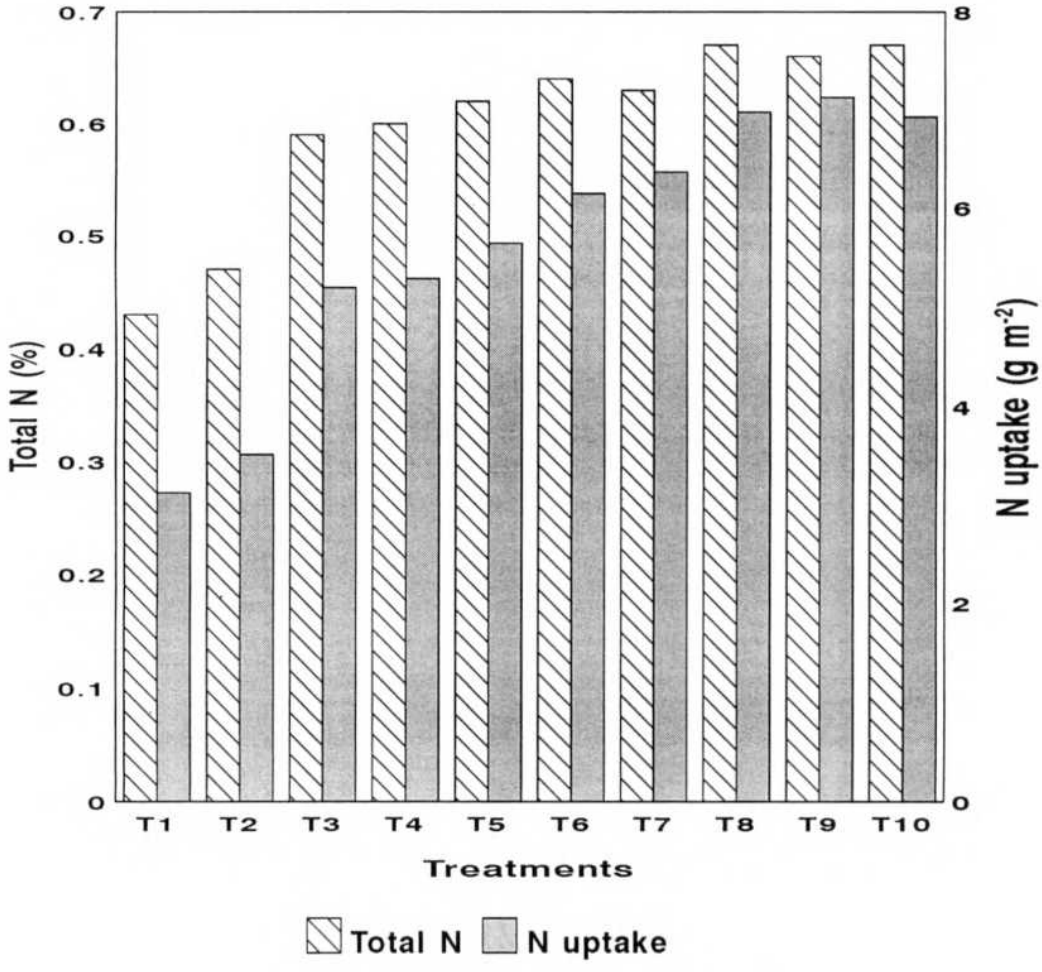
4.6.4. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the total N and N uptake by ADT 36 rice.

The results are presented in Table 26 and Fig. 22. Cyanobacterial inoculation significantly improved the total N content and N uptake by the ADT 36 rice. The combined application of the composite culture of the herbicide tolerant cyanobacterial isolates and 75 per cent of the recommended N registered the highest total N content. Similar trend was seen in N uptake, which was on par with 100 per cent N application. The results clearly proved the ability of the herbicide tolerant cyanobacterial isolates to improve the total N and N uptake by the ADT 36 rice crop.

Table 26. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the total N content and N uptake of ADT 36 rice

Treatment	Total N at harvest (%)	% increase over control	N uptake ($g\ m^{-2}$)	% increase over control
T_1 - H_0 I_0 N_0	0.43	-	3.11	--
T_2 - H_0 I_1 N_0	0.47	9.30	3.57	14.79
T_3 – H_1 I_0 N_0	0.59	37.20	5.19	66.88
T_4 – H_1 I_1 N_0	0.60	39.5	5.28	69.77
T_5 - H_0 I_0 (75% N)	0.62	44.18	5.64	81.35
T_6 - H_0 I_1 (75% N)	0.64	48.83	6.14	97.42
T_7 - H_1 I_0 (75% N)	0.63	46.51	6.36	104.50
T_8 – H_1 I_1 (75% N)	0.67	55.81	6.98	124.43
T_9 – H_1 I_0 (100% N)	0.66	53.48	7.13	129.00
T_{10} – H_1 I_1 (100% N)	0.67	55.81	6.93	122.80
SEd	0.02		0.23	
CD	0.05		0.49	

Fig 22. Effect of inoculation of the herbicide tolerant cyanobacterial isolates along with graded levels of N on the total N content and N uptake of ADT 36 rice



T_1 - H_0 I_0 N_0
T_2 - H_0 I_1 N_0
T_3 - H_1 I_0 N_0
T_4 - H_1 I_1 N_0
T_5 - H_0 I_0 N75
T_6 - H_0 I_1 N75
T_7 - H_1 I_0 N75
T_8 - H_1 I_1 N75
T_9 - H_1 I_0 N100
T_{10} - H_1 I_1 N100

DISCUSSION

5. DISCUSSION

Cyanobacteria are photoautotrophic organisms capable of doing both photosynthesis and N_2 fixation simultaneously. They are probably the largest and most diverse group of prokaryotes. They posses chlorophyll-a which distinguishes them from the other photosynthetic bacteria. Besides chlorophyll-a, cyanobacteria have phycobiliproteins. Cyanobacteria are ubiquitous in nature and are found in extreme environments. They have very good mechanisms to adapt to the extremes of temperature, desiccation, illumination, radiation, salinity, pH, toxicants and scarcity of nutrients. This probably accounts for their diverse occurrence from the barren desert soils to the fertile rice soils.

The predominance of cyanobacteria in the rice ecosystem has been well documented. Reports are available on the predominance of cyanobacteria in various ecosystems *viz.*, normal wetland rice soils (Kannaiyan, 1990), saline soils (Thomas and Apte, 1984; Amsaveni and Kannaiyan, 1995) and acid soils (Aiyer, 1965; Madhusoodhanan and Dominic, 1995; Tamilselvam, 1998).

Cyanobacteria play a major role in maintaining or even improving the fertility status of rice fields (Singh, 1961). The dominant genera in the rice fields are *Anabaena, Anabaenopsis, Aulosira, Cylinderospermum, Scytonema, Tolypothrix, Fischerella, Haphalosiphon, Mastigocladus, Stigonema, Westiellopsis, Westiella* and *Campylonema.* The beneficial role of cyanobacteria extends from nitrogen fixation to the production of growth promoting substances (Gupta and Shukla, 1972; Merina Premkumari and Kannaiyan, 1994), vitamins and aminoacids (Okada and Yamaguchi, 1955).

Pesticides and herbicides are the important inputs in the present day intensive rice culture. To get the desired response of cyanobacteria it is very much essential to develop tolerant strains to those agrochemicals. The earlier studies indicated the

effect of these agrochemicals on soil flora and fauna (Grossbard, 1976). The agrochemical usage in India rose sharply from a mere 5000 t. in 1961 to 68,000 t. in 1991 (Adhikary, 1998). Presently, 51755 t. of insecticides, 22895 t. of fungicides and 7620 t. of herbicides are used in India (Husmani, 1998).

The rice field herbicides include the phenoxys (2,4-D, MCPA), the amides (propanil, butachlor), thiocarbamates (thiobencarb), dinitroanilines (trifluraline), pyrimidines (bentazon) and dithiophosphates (anilophos). Among the rice field herbicides, butachlor is the most popular and widely used for lowland rice.

Any external application of synthetic nature would have a direct influence on the soil flora and fauna. This was experimentally proved by several investigators and concluded that herbicide application in the long run may disturb the biological equilibrium in the soil (Selvamani and Sankaran, 1993; Gopalaswamy *et al.*, 1994). Most of the herbicides when used clearly inhibited the photosynthetic oxygen evolution (Singh and Tiwari, 1988).

Inger (1970) noticed that the field application of 2,4-D and MCPA at the recommended levels inhibited the growth and nitrogen fixation by cyanobacteria. Shivaram and Shivappa Shetty (1988) observed a favourable effect with pesticides at low concentration on the growth and nitrogen fixation by cyanobacteria. The growth and metabolic activity of cyanobacteria were adversely affected by several pesticides used in agriculture and thereby poses a major threat to soil fertility (Venkateswaralu, 1993).

Both herbicide application and cyanobacterial inoculation are mostly synchronized through soil application. There were reports on the ill effects of herbicides to soil microorganisms (Gopalaswamy *et al.*, 1994) as well as cyanobacteria (Zagar and Dar, 1990).

Much of the previous studies involved the use of standard unicellular cyanobacterial isolates obtained from the American Type Culture Collection, USA to study the herbicidal effects. Under field conditions, it is highly essential to isolate the cultures from a particular environment i.e., soils amended with herbicides or permanently exposed to herbicides. If cyanobacterial cultures are isolated from such environments their performance will be much better in the herbicide applied situation. With this aim several cyanobacterial cultures were isolated from the soils collected from the permanent herbicide amended experimental plots and were purified using antibiotics.

5.1. ISOLATION AND CHARACTERIZATION OF CYANOBACTERIA FROM HERBICIDE AMENDED SOILS

Several techniques are available for the isolation of cyanobacteria with ease under laboratory conditions. They include direct inoculation into minimal media, soil dilution and moist culture techniques. Isolation of axenic cultures can be achieved by washing with chlorine water (Fogg, 1942) detergents and phenols (McDaniel *et al.*, 1962) and antibiotics (Fogg *et al.*, 1973). Among the methods antibiotic treatment was found to be highly successful.

In the present study, cyanobacterial cultures were isolated from herbicide amended rice soils and purified using antibiotics. The dominant flora were *Westiellopsis, Anabaena, Nostoc* and *Oscillatoria.* Besides, unicellular cyanobacteria such as *Microcystis* and *Synechococus* were also present in minor proportions.

Upon purification the cyanobacterial isolates were characterized based on growth, biochemical constituents *viz.*, chlorophyll-a content, phycobiliproteins, ammonia excretion pattern and protein content. The growth and biochemical constituents were maximum in *Westiellopsis* -HT-SGK-1 and *Westiellopsis* -HT-SGK-2 followed by *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-2, *Oscillatoria*-HT-

SGK-1, and *Anabaena*-HT-SGK-1. The *Westiellopsis* isolates also showed maximum ammonia excretion.

The predominance of *Westiellopsis* in problem soils such as saline soils (Amsaveni and Kannaiyan, 1995), acid soils (Tamilselvam, 1998) has been well documented. In herbicide amended soils the predominance of *Westiellopsis* might be due to the suppression of the other genera. This has been demonstrated by several workers that pesticides stimulate certain cyanobacterial genera while suppressing certain others (Adhikary, 1998). The results of the present study are in accordance with the earlier findings.

5.2. EFFECT OF BUTACHLOR ON THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES

Butachlor (N- (butoxy methyl) 2-Chloro-2'6' diethyl acetanilide) is a selective pre-emergence herbicide used for weed control in rice. It belongs to the acid amide group of herbicides. Among the different cyanobacterial isolates, *Westiellopsis* isolates were found to tolerate butachlor even at the highest concentration of 12 ppm which is more than the field level application. *Westiellopsis* -HT-SGK-1 and *Westiellopsis* -HT-SGK-2 did not show any reduction in growth and biomass production. However there was no inhibition to *Anabaena*-HT-SGK-2 at 12 ppm initially but a slight reduction in growth and biomass was noticed in the later stages. The growth and biomass production of the cyanobacterial isolates *Anabaena*-HT-SGK-1, *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1 were inhibited by butachlor at all concentrations.

Though the chlorophyll-a content of the cyanobacterial isolates was inhibited by butachlor in general, it was not affected even at 12 ppm in the case of *Westiellopsis* -HT-SGK-1 and *Westiellopsis* -HT-SGK-2. Interestingly butachlor was found to be stimulatory to *Anabaena*-HT-SGK-2 at 9 ppm concentration. The chlorophyll-a content of all other cyanobacterial cultures *viz.*, *Anabena* HT-SGK-1,

Nostoc-HT-SGK-1, and *Oscillatoria*-HT-SGK-1 was inhibited by butachlor at all concentrations at all the three incubation periods.

Meeks *et al.*, (1985) revealed that nearly 58 per cent of the 66 per cent of the ammonia acquired for the total radioactive compounds formed on incubation of *Anabaena* preparations with $^{13}N2$ was in the extracellular fraction. About 35 per cent of the $^{15}N_2$ fixed by the isolated cyanobacteria symbionts was released in the medium as ammonium (Peters *et al.*, 1980).

The ammonia excretion pattern by the cyanobacterial isolates as influenced by different concentrations of butachlor was assessed *Westiellopsis* -HT-SGK-1 and *Westiellopsis* -HT-SGK-2 were able to excrete maximum amount of ammonia in the growth medium even at the highest concentration of 12 ppm. Interestingly these two isolates registered maximum pigment production even at the highest concentration of butachlor *viz.*, 12 ppm.

The effect of butachlor on cyanobacteria was studied by several investigators. Careful perusal of the findings clearly led to the conclusion that there exists a variation among the cyanobacterial cultures to butachlor. Kashyap and Pandey (1982) observed maximum tolerance of *Anabaena dolinum* to butachlor. While Roychoudhry and Kaushik (1986) observed that the growth and chlorophyll synthesis of *Tolypothrix ceylonica* and *Scytonema cincinnatum* was not affected by 10 ppm of butachlor. Kolte and Goyal (1992) observed that *Anabaena khannae* and *Calothrix marchia* were tolerant to butachlor. Rather (1994) observed that butachlor at 0.005 mg ml^{-1} gave an initial increase in growth compared to control.

Much of the earlier studies involved the use of cyanobacteria from culture collections. Since this study showed that *Westiellopsis* was the predominant flora in herbicide amended soils, it has the natural inherent mechanism to perform even at higher concentration of butachlor. The variations observed among the

cyanobacterial cultures isolated from herbicide amended soils to different concentrations of butachlor are in agreement with the earlier findings.

Singh *et al.* (1986) observed that butachlor has no adverse effect on cyanobacteria even at increased concentration. The growth and biomass production were maximum in *Westiellopsis* -AT-TGK-4A7 even at 10 ppm concentration of butachlor (Tamilselvam, 1998). Singh and Vaishampayan (1978) observed growth inhibition in *Nostoc muscorum* at 150 ppm of butachlor but the present study clearly showed that there was a clear cut inhibition of the *Nostoc* isolate even at 3 ppm. This might be due to the variation at the species level.

The chlorophyll content of the cyanobacterial cultures *viz.*, *Anabaena* and *Nostoc* were progressively inhibited with increasing concentrations of butachlor (Veena Nagpal and Goyal, 1992). The results of the present study are in agreement with the above findings.

Ammonia excretion by the herbicide tolerant cyanobacterial isolates revealed variation among the cyanobacterial isolates. This was in agreement with the findings of Subramaniam and Shanmugasundaram (1986) who observed a variation in the ammonia excretion by *Anabaena* under the influence of 2, 4-D.

Phycobiliproteins, the antenna pigments are assembled into macromolecular aggregates called phycobilisomes (Glazer, 1987) and can be divided into three major classes *viz.*, C-phycoerythrin, C-phycocyanin and allophycocyanin. The phycobiliproteins of heterocystous cyanobacteria are associated primarily with photosystem II in vegetative cells and are absent in heterocysts that harbour only PS1 (Haselkorn, 1978).

In the present study, a 50 per cent reduction in the phycobiliproteins was observed in the isolates *viz.*, *Anabena* -HT-SGK-1, *Nostoc*-HT-SGK-1 and

Oscillatoria-HT-SGK-1. Similar reduction in the phycobiliproteins of *Anabaena dolionum* was observed by Kashyap and Pandey (1982).

Proteins form a major component of cyanobacteria contributing 1.9 per cent of dry weight and 90 per cent of nitrogen (Show, 1981), Proteins are the major component in the C/N ratio and vary with the nitrogen availability (Jackson, 1980; Ryther and Hanisak, 1982).

Reduction in the protein content of the cyanobacterial isolates *viz.*, *Anabaena* HT-SGK-1 and *Oscillatoria*-HT-SGK-1 was observed at 3 ppm of butachlor. While *Westiellopsis* -HT-SGK-1 and *Westiellopsis* -HT-SGK-2 did not show any marked reduction in the protein content even at 12 ppm of butachlor. The above findings are in conformity with the results obtained by Kashyap and Pandey (1982) who observed a decline in the protein content of *Anabaena dolionum* with 5 ppm butachlor.

5.3. EFFECT OF BUTACHLOR ON NITROGEN FIXATION BY THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES

Nitrogen fixation in cyanobacteria is achieved through the enzyme nitrogenase which is localized in specialized cells called heterocysts. Over 125 strains of cyanobacteria of both heterocystous and non heterocystous are known to fix nitrogen (Stewart *et al.*, 1979). Nitrogen starved cyanobacteria like *Nostoc, Anabaena, Oscillatoria* and *Anacystis* grown in the presence of excess carbon accumulate glycogen (Ernst *et al.*, 1984). This provides a carbon pool for protein synthesis and source of reductant needed for the enzymatic conversion of acetylene to ethylene (Rippka and Waterburry, 1977; Ernst *et al.*, 1984).

In the present investigation the isolates *viz.*, *Westiellopsis* -HT-SGK-1 and *Westiellopsis* -HT-SGK-2 registered maximum nitrogenase activity even in the presence of butachlor. The inhibition of the nitrogenase activity was more in the

isolates *viz.*, *Anabaena*-HT-SGK-1, *Anabaena*-HT-SGK-2, *Nostoc*-HT-SGK-1 and *Oscillatoria*-HT-SGK-1 even with 3 ppm concentration.

The findings are in accordance with the results of Kashyap and Pandey (1982) who observed inhibition of heterocyst differentiation and nitrogenase activity by butachlor at 5 ppm in *Anabaena*. The above results suggest the negative influence of herbicides on the nitrogenase activity of cyanobacteria.

5.4. MOLECULAR CHARACTERIZATION OF THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES

Cyanobacteria are known to harbour both chromosomal and extrachromosomal forms of DNA. Cyanobacterial plasmids were first described by Asato and Guinoza (1973) in the cyanobacterium *Anacystis nidulans*. Since then endogenous plasmids have been reported in various typological groups. Their number may vary from one to many with 1.3 kb to 130 kb size (Goyal, 1998).

In the present study, an attempt was made to screen the herbicide tolerant cyanobacteria for the presence of plasmids. This was done by extracting the total DNA during the exponential growth phase and running the DNA in an agarose gel.

The results of the present study have shown that plasmids are not present in any of the herbicide tolerant cyanobacterial isolates and only the chromosomal DNA was present. This was evident from the single intact band that appeared at the position corresponding to the 23 kb band of the marker. This was similar in all the cases.

Though plasmids are available in cyanobacteria their role in physiology, ecology and evolution is not known (Goyal, 1998). The role of plasmids has been hypothesized by several investigators, but not clear. It could be concluded that the herbicide tolerance genes in the cyanobacterial isolates are not plasmid borne.

5.5. EFFECT OF INOCULATION OF THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES ON ADT 36 RICE SEEDLINGS

Considerable interest has been generated over the past few decades to exploit the nitrogen fixing potential of the micro organisms. The cyanobacteria are known to excrete ammonia in rice fields both under free living and symbiotic conditions. Besides they also produce a variety of growth promoting substances which stimulate the seed germination and seedling growth of rice (Kannaiyan, *et al.*, 1985). With herbicides becoming an integral part of the package of lowland rice cultivation, it is necessary to develop tolerant strains of cyanobacteria that are not affected by field level concentrations of the applied herbicides.

In the present study, the inoculation of the herbicide tolerant cyanobacterial isolates increased the plant height, chlorophyll content and total nitrogen content of ADT 36 rice seedlings significantly over control. The ammonia excretion in flood water was also higher in the inoculated treatments. The composite culture inoculation significantly increased all the above parameters both in the presence and absence of butachlor. Among the individual treatments *Westiellopsis* -HT-SGK-2 inoculation registered maximum plant growth, total chlorophyll content, total nitrogen content and flood water ammonia.

Similar observation in improving the growth, chlorophyll content and total nitrogen content of rice due to the inoculation of the composite culture was observed by Tamilselvam (1998).

The improved performance of the inoculated seedlings might be due to the production of growth promoting substances and the continuous photoproduction of ammonia leading to enhanced nutrient availability (Tamilselvam, 1998).

5.6. EFFECT OF INOCULATION OF THE HERBICIDE TOLERANT CYANOBACTERIAL ISOLATES ALONG WITH GRADED LEVELS OF N ON THE YIELD OF ADT 36 RICE

Cyanobacteria have been used as a biofertilizer for rice crop for decades and the effect of the cyanobacterial inoculation on the yield of rice has been well established by a number of workers (Kannaiyan, 1990). The nitrogen fixed by the cyanobacteria is made available to rice crop through exudation or microbial decomposition after the death of the cyanobacteria (Santra, 1993). The nitrogen contribution by cyanobacteria has been put at 14 - 40 Kg ha^{-1} by several workers (De and Mandal, 1956; Sankaram 1971; Yoshida and Ancaja, 1973).

In the present study, composite cultures of herbicide cyanobacteria were inoculated with graded levels of N both in the presence and absence of butachlor. Cyanobacterial inoculation combined with 75% of recommended N and butachlor at the recommended dosage improved the plant biometrics and yield attributes of ADT 36 rice. While cyanobacterial inoculation alone in the absence of N application did not show any significant effect on the yield of ADT 36 rice. However maximum grain yield was obtained with cyanobacterial inoculation along with 75% N and butachlor application. The straw yield was also significantly higher. Significant increase in the total N content and N uptake of ADT 36 rice was observed due to the combined inoculation of the composite inoculum along with 75% N in the presence of butachlor.

Venkataraman (1979) observed that algalization could save upto 25 - 30 kg N fertilizer without any reduction in the rice crop yield. Kannaiyan *et al.* (1982) demonstrated that the effect of top dressing of fertilizer nitrogen did not affect the establishment and N_2 fixation by the inoculated cyanobacteria.

Pachpande (1990) observed that the combined application of urea and cyanobacteria was more effective than application of urea alone. Chirriv *et al.* (1995) observed that the performance of paddy crop fertilized with 100 kg N ha^{-1} was comparable with 50 Kg N ha^{-1} combined with 20 kg BGA inoculation. While Singh *et al.* (1995) obtained maximum rice yield with 80. Kg N ha-1 as USG along with cyanobacterial inoculation which was comparable to the application of 101 kg N ha^{-1} as prilled urea alone.

The results of the present study also indicated the trends observed by the earlier workers. N savings of upto 25 per cent can be obtained due to the inoculation of the herbicide tolerant cyanobacterial isolates even in the presence of recommended dosage of butachlor.

The results of this study clearly showed that the herbicide tolerant isolates performed better in increasing rice yield even in the presence of butachlor. The efficacy of inoculation could be enhanced with composite culture inoculation along with 75 per cent of recommended N fertilizer.

SUMMARY

6. SUMMARY

The use of agrochemicals in rice production is an integral part of intensive agriculture. In modern day agriculture herbicide usage is increasing manifold. Any external application of agrochemicals especially herbicides to soil would have a direct bearing on soil microorganisms, including cyanobacteria which play a vital role in N_2 in rice productivity. Though the cyanobacterial inoculation has been advocated for the past several decades the desired response to inoculation is hampered due to various reasons like application of poor quality soil based inoculum, non use of location specific cultures and few attempts to isolate location specific stress tolerant cultures. To overcome this concerted efforts are made to develop efficient cyanobacterial cultures which could thrive well in extreme environments such as saline soils, acid soils, etc. Because of the frequent use of herbicides in rice cultivation this study has been planned to develop-efficient cyanobacterial cultures to tolerate the herbicide concentration to the maximum extent and to use them as an effective biofertilizer for rice.

In the present investigation attempts were made to isolate cyanobacterial cultures from the soils permanently amended with herbicides such as butachlor and 2, 4-D. Thirteen cyanobacterial isolates were isolated and purified using triple antibiotics. Six cyanobacterial isolates *viz.*, *Anabaena* - HT-SGK-1, *Anabaena* - HT-SGK-2, *Nostoc*-HT-SGK-1, *Oscillatoria*-HT-SGK-1, *Westiellopsis*- HT-SGK-1 and *Westiellopsis* - HT-SGK-2 were selected and tested for their growth, biomass production and biochemical constituents.

The herbicide tolerance of these cyanobacterial isolates was tested by growing them in BG-11 medium supplemented with butachlor at various concentrations *viz.*, 3, 6, 9 and 12 ppm. BG-11 medium without butachlor served as control. Further the molecular characterization of the cyanobacterial isolates was

done by screening for the presence of plasmids. The biofertilizer effect of these cyanobacterial isolates was tested in pot culture upto the seedling stage in ADT 36 rice by inoculating them either individually or in composite culture both in the presence and absence of butachlor. Finally the performance of these cyanobacterial isolates under graded levels of N fertilizer was tested in ADT 36 rice. The growth and yield parameters were assessed. The results are summarized below.

1. Thirteen cyanobacterial isolates belonging to the genera *Anabaena, Nostoc, Oscillatoria* and *Westiellopsis* were isolated from herbicide amended rice soils. The dominant genus was *Westiellopsis*.

2. *Westiellopsis*-HT-SGK-1 and *Westiellopsis*-HT-SGK-2 performed better by registering maximum growth, biomass production. The chlorophyll-a content, pigment production, ammonia excretion and protein content were higher in *Westiellopsis*-HT-SGK-2 followed by *Westiellopsis*- HT-SGK-1 and *Anabaena*-HT-SGK-2.

3. In general there was a linear inhibition of growth, biomass production and biochemical constituents of the cyanobacterial isolates to increased concentrations of butachlor. Maximum inhibition by butachlor was noticed on 10th day after inoculation. Interestingly though a slight inhibition was noticed at a lower concentration of butachlor, it was stimulatory to *Westiellopsis* at higher concentrations.

4. Both *Westiellopsis* isolates *viz.*, HT-SGK-1 and HT-SGK-2 have shown higher growth, biomass, chlorophyll content, ammonia excretion, phycobiliprotein content at 12 ppm of butachlor. In the case of *Anabaena*-HT-SGK-2, butachlor was stimulatory upto 9 ppm only. For the other isolates butachlor was inhibitory.

5. The N_2 fixing capacity of the herbicide tolerant cyanobacterial isolates was assessed. There was inhibition to nitrogenase activity due to butachlor. Among the cyanobacterial isolates *Westiellopsis* showed maximum nitrogenase activity even in the presence of butachlor.

6. The agarose gel electrophoresis revealed the presence of a single band of chromosomal DNA. No plasmids were detected in any of the herbicide tolerant cyanobacterial isolates.

7. Inoculation of the composite inoculum at 1g fresh weight tub^{-1} increased the seedling height, chlorophyll content and total N over individual inoculation both in the presence and absence of butachlor.

8. For getting the desired inoculation effect in the presence of the herbicide, inoculation of a composite inoculum along with 75% of the recommended N as urea was the best. This treatment gave significantly increased plant growth and yield parameters on par with 100% N application.

REFERENCES

REFERENCES

Abe, T., M.Tsuzuki, Y.Kadokani and S.Miyach. 1988. Isolation and characterization of temperature sensitive, high CO_2 requiring mutant of *Anacystis nidulans* R2. **Plant Cell Physiol., 29**: 1353 - 1360.

Adhikary, S.P. 1998. Interaction of cyanobacteria with pesticides. In: **Advances in phycology** (eds.) B.N.Verma, A.N.Kargupta. and S.K.Goyal, APC Publication, New Delhi. p. 221 - 250.

Ahluwalia, A.S., Reena and Dahuja, S. 1993. Studies on the role of algalization on growth and yield of rice (*Oryza sativa* Linn.). **Res. Bull. of the Punjab Univ., 43**:55-60.

Ahmad, M.H. and G.S.Venkataraman. 1973. Tolerance of *Aulosira fertilissima* to pesticides. **Curr. Sci., 42**: 108 - 110.

Aiyer, R.S. 1965. Comparative alagalogical studies in rice fields of Kerala state. **Agric. Res. J. Kerala 3**(1): 100-111.

Amla, D.V. 1979. Characteristics of pigment mutants of *Anacystis nidulans* : Ultraviolet sensitivity and multiplication of cyanophage AS-1. **Biochem. Physiol. Plant., 174**: 678 - 684.

Amsaveni, P. 1995. Effect of certain mutagens on the growth and ammonia excretion by the saline tolerant cyanobacteria and their role as biofertilizer for bioreclamation of saline and sodic soils. Ph.D. Thesis, Tamil Nadu Agrl. Univ., Coimbatore, Tamil Nadu, p.276.

Amsaveni, P. and S. Kannaiyan. 1995. Influence of nitrogen and phosphorus sources on the growth, nitrogen fixing activity and ammonia excretion of salt tolerant cyanobacteria. In : **National Seminar on *Azolla* and Algal Biofertilizers for Rice** (ed.) S. Kannaiyan, Tamil Nadu Agri. Univ. Publ., Coimbatore, Tamil Nadu, India, pp.10.

Anand, N. 1989. Hand book of Blue Green Algae of rice fields of South India. Bishen Singh Mahendra Pal Singh. Dehradun. p. 78.

Anand, N., R.S.S. Hopper and G. Jagatheswari. 1994. Response of certain blue green algae (Cyanobacteria) to salinity. In: Recent Advances in phycology

(eds.) A.K. Kashyap and H.D. Kumar, Rastogi Pub, Varanasi, India, pp. 125-129.

Apte, S.K. and J. Thomas. 1984. Effect of sodium on nitrogen fixation in *Anabaena torulosa* and *Plectonema boryanum*. **J. Gen. Microbiol., 130**: 1161-1168.

Asato, Y. and Guinoza, H.S. 1973. Separation of small circular DNA molecules from the blue green alga *Anacystis nidulans*. **Nature New. Biol., 244**: 132-133.

Avrick, J.H., D.L.Wilson and L.C.Darlington. 1971. Response of soil algae to pichloram - 2,4-D mixtures., **Weed Sci., 19**: 276.

Bennet, A. and L. Bogorad. 1971. Properties of subunits and aggregates of blue green algal biliproteins. **Health Lab Sci.,3**: 90 - 100.

Bogorad, L. 1975. Phycobiliproteins and complementary chromatic adaptation. **Ann. Rev. Plant. Physiol., 26**: 369 - 401.

Boussiba, S. and J. Gibson. 1991. Ammonia translocation in cyanobacteria. **FEMS Microbiol. Rev., 88**: 1-14.

Brock, T.D. 1973. Lower pH limit for the resistance of blue green algae. Evolutionary and ecological implications. **Science, 179**: 480-483.

Bryant, D.A.,G.Gohen-Bazire and A.N.Glazer. 1981. Characterization of the biliproteins of *Gloeobacter vidaceus*. **Arch. Microbiol., 129**: 190 - 198.

Bryant, S.A., A.N.Glazer and F.A.Eiserling. 1976. Characterization and structural properties of major biliproteins of **Anabaena** sp. **Arch. Microbiol., 110**: 61 - 75.

Carr, N.G. and B.A.Whitton. 1982. **The Biology of Cyanobacteria**. Blackwell Scientific Publications, Oxford. pp.1-688.B.

Cerniglia, C.E., D.T.Gibson and C.VanBaalen. 1979. Algal oxidation of aromatic hydrocarbons : formation of 1 - napthol from napthalene by *Agmenellum quadruplicatum* strain PR-6. **Biochem. Biophys. Res. Commun.,** 77:50-59.

Chao, J. and C.C. Bower. 1971. Purification and properties of glycogen isolated from blue green algae, *Nostoc muscorum*. **J. Bacteriol., 104**: 334-338.

Chirriv, A.J, G.M. Borkar and V.W. Tarsekar. 1995. Nitrogen fixation by blue green algae and fertility of paddy soils. **J. Soils and Crops.,** 4(2): 169-171.

Cullimore, D.R. and A.E.McCann. 1977. Influence of four herbicides on the algal flora of a prairie soil. **Plant and Soil. 46**: 455-459.

De, P.K. 1939. The role of blue green algae in rice fields. **Proc. Res. Soc. London., 127**: 121 - 139.

De, P.K. and L.N. Mandal. 1956. Fixation of nitrogen by algae insoils. **Soil Sci., 81**:453.

Demming-Adams, B. and W.W. Adams. 1992. Photoprotection and other responses of plants to light stress. **Ann. Rev. Plant Physiol. Plant Mol. Biol., 43**: 599 - 626.

Desikachary, T.V. 1959. **Cyanophyta**. Indian Council Agri. Res. New Delhi.

Drews, K. 1928. Uber die Assimilation des Luftsticksloffs durch Baludgen. **Zbl. Back. Abt., 76**: 88-121

Ernst, A., H. Kirschenlohr, J. Diez and P. Boger. 1984. Glycogen content and nitrogenase activity in *Anabaena variabilis*. **Arch. Microbiol., 140**: 120-125.

Erokhina, L.B. 1990. Accumulation of phycobiliproteins in cells of free living nitrogen fixing cyanobacteria growing on different nitrogen sources. **Soviet Plant Physiol., 37**: 873 - 879.

Fogg, G.E. 1942. Studies on nitrogen fixation by blue green algae. **F. Brit. J. Expt. Biol., 19**:78-87.

Fogg, G.E., W.D.P. Stewart, P. Fay and A.E. Walsty. 1973. **The Blue Green Algae**. Academic Press, London, pp.459.

Fogg, G.E. 1949. Growth and heterocyst production of *Anabaena cylindrica* Lemn. 11. In relation to carbon and nitrogen metabolism. **Ann. Bot., 13**: 241-259.

Fogg, G.E., W.D.P. Stewart, P. Fay and A.E. Walsby. 1949. The Blue Green Algae. Academic Press, London, pp. 459.

Fonkes, A.G., M.A. Vargas, I. Moreno, M.G. Guerrero and M. Losada. 1987. Factors affecting the production of biomass by an nitrogen fixing blue green algae in outdoor culture. **Biomass, 13**: 33-43.

Gallon, I.R. 1980. **Nitrogen fixation by photoautotrophs**. In: Nitrogen Fixation. (eds) W.D.P. Stewart and J.R. Gallon, Academic Press, London, pp.197-238.

Gamble, S.J.R., C.J. Maybew and W.E. Chappel. 1952. Respiration rates and plate counts for determining the effect of herbicides on heterotrophic soil micro organisms. **Soil. Sci., 74**: 347-352.

Gantl, E. 1980. Structure and function of phycobilisomes : light harvesting pigment complexes in red and blue algae. **Intl. Rev. Cytol., 66**: 45 - 80.

Ghosh, T.K. and K.C. Saha. 1993. Effect of inoculation of nitrogen fixing cyanobacteria on the nitrogenase activity in soil and rhizosphere of wetland rice. **Biol. Fertil. Soil., 16**: 16-20.

Glazer, A.N. 1977. Phycobilisomes : assembly and attachment. In: **The cyanobacteria**. (eds). P. Fay, C.Van Baleen, Elsevier Pub., Amsterdam. pp. 69 - 74.

Glen, W.S. 1984. Effect of herbicide atrazine and its degradation products alone and in combination with phototrophic microorganisms. **Arch. Environ. Contam. Toxicol., 13**: 35 - 42.

Gopalaswamy, G., S.Anthoniraj and A. Abdul Kareem. 1994. Interaction of herbicides with **Azolla** and soil microbes. **Indian J. Weed Sci., (3 and 4)**: 28 - 34.

Goyal, D. 1998. Cyanobacterial cloning systems. In:**Advances in phycology** (eds.) B.N. Verma, A.N. Kargupta and S.K. Goyal, APC Publication, New Delhi, p. 221-250.

Goyal, S.K. and G.S Venkataraman. 1971. Response of high yielding rice varieties to algalization. Interaction of soil types with algal inoculation. **Phykos, 10**: 32-33.

Gregory, W.W., J.K. Reed and L.E.Priester. 1969. Accumulation of parathion and DDT by some algae and protozoa. **J. Protozol., 16**: 69 - 72.

Grossbard, E. 1976. Effect of herbicides on the soil microflora. In : Herbicides, physiology, biochemistry, ecology (ed.) J.J.Andus, Academic Press, London, pp.99.

Grossbard, E. and H. A. Davies. 1976. Specific microbial response to herbicides. **Weed Res., 16**: 163-169.

Guerrero, M.G., J.L. Ramos and M. Losada. 1982. Photosynthetic prodution of ammonia. **Experientia, 38**: 53-58.

Gupta, A.B. and A.C. Shukla. 1972. Studies on the nature of algal growth promoting substances and their influence on growth, yield and protein content of rice plants. **Labdev. J. Sci. Technol., 5**:163-163.

Gustavson, K. and S.A. Wanberg. 1995. Tolerance induction and succession in micro algae communities exposed to copper and atrazine. **Aquatic Toxicology, 32**(4):283-302.

Hallenbeck, P. C. and J.R. Benemann. 1980. In: **Nitrogen fixation (Enzymology, physiology, genetics) - Application in H_2 and NH_3 production** (ed.) P.M. Viganais, Grenolbe. Abstracts of a societe de chimie Biologique commission of the European community meeting a nitrogen fixation.

Hardy, R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns. 1968. The acetylene reduction assays for N_2 fixation. Laboratory and field evaluation. **Plant Physiol., 43**: 1185 - 1207.

Haselkorn, R. 1978. Heterocysts. **Annu. Rev. Plant Physiol., 29**: 319 - 344.

Hauxby, K., B. Tubea, J. Ownby and E. Baseler. 1977. Effect of various herbicides on four species of algae. **Pest. Biochem. Physiol., 7**: 203.

Huang, T.C. and T.T. Chow. 1986. New types of N_2 fixing unicellular cyanobacterium (blue green algae). **FEMS Microbiol. Lett., 36**: 109-110.

Humpries, E.C. 1956. Mineral composition and ash analysis. In: **Modern Methods of Plant Analysis**. (eds.) K. Peach and M.V.Tracy. Vol.I, Springer Verlag. Berlin, pp. 468 - 502.

Husmani, M.M. 1998. Usage of pesticides and herbicides. **Agro India, 23**(4):10-11.

Ibrahim, A.N. 1972. Effect of certain herbicides on growth of nitrogen fixing algae and rice plants. **Symp. Biol. Hung., 11**: 445-448.

Inger, L. 1970. Effect of two herbicides on nitrogen fixation by blue green algae. **Sov. Bot. Tidskr., 64**:468-502.

Ishizawa, S. and T. Matsuguchi. 1966. Effects of pesticides and herbicides upon microorganisms in soil and under waterlogged condition. **Bull. Nati. Inst. Agr. Sci. Tokyo., 16**: 1 - 7.

Islam, M.R. and B.A. Whitton. 1992. Cell composition and nitrogen fixation by the deep water rice field cyanobacteria *Calothrix* D 764. **Microbiol., 69**(278): 72-88.

Jackson, G.A. 1980. Marine biomass production through sea and weed aquaculture. In : **Biochemical and photosynthetic aspects of energy production** (ed.) A. San Pietro, Academic Press, pp.31-58.

Jaganathan, R. and S. Kannaiyan. 1977. Effects of blue green algae application on rice yield. **Intl. Rice Res. Newslett., 3**(4): 20.

Kannaiyan, S., M. Thangaraj and G. Oblisami. 1982. Effect of combined inoculation of blue green algae with *Azolla* and *Azospirillum* on rice crop. **BNF Newslett., 1**(2): 32.

Kannaiyan, S. 1978. Mass scale multiplication on blue green algae under field condition. **All India Seminar on Blue green algae and their viruses**. Madurai Kamaraj University, Madurai, p. 32 (Abstr).

Kannaiyan, S. 1983. Studies on the effect of algal biofertilizer inoculation on rice crops. Paper presented in **National Conf. Society. Basic and Appl. Microbiologists**. Dunger College, Bikaner, India.

Kannaiyan, S. 1985. Studies on the algal application for low land rice crop. **Tamil Nadu Agri. Univ. Bull.**, Coimbatore, Tamil Nadu.

Kannaiyan, S. 1990. Blue green algal biofertilizers. In : **Biotechnology of Biofertilizers for Rice Crop**, (ed.) S. Kannaiyan, Tamil Nadu Agri. Univ. Publ., Coimbatore, India, pp.212-225.

Kashyap, A.K. and K.D. Pandey. 1982. Inhibitory effects of the rice field herbicide machete on *Anabaena dolionum* Bharadwaja and protection by nitrogen sources. **Z. Pflanzenphysiol., 107**: 339 - 345.

Kaushik, B.D. 1987. Blue green algae, its role in salt affected soils. **International symposium on phycology** , Madras, V.20, p.56 (Abstr.) .

Kitchen, L.M., W.W.Witt and C.C.Rieck. 1986. Inhibition of chlorophyll accumulation by glyphosphate. **Weed Sci., 29**:513-516.

Kolte, S.O. and S.K. Goyal. 1992. On the effect of herbicides on growth and nitrogen fixation by cyanobacteria. **Acta. Bot. Indica., 20**: 225 - 229.

Komarek, J. 1998. Studies on the cyanophytes of Cuba. **Folia. Glo-Bot. Phytotaxon., 30(1)**: 81 - 90.

Koyama, Y. 1991. Structure and function of carotenoids in photosynthetic systems. **J. Photochem. Photobiol., 9**: 265 - 280.

Lales, J.S., M.A. Lapitan and R.S. Matrl. 1989. Response of *Azolla* to herbicides. In: ***Azolla*: Its culture management and utilization in the Philippines,** National *Azolla* Action Programme. UPLB, Los Banos, Philippines. p.91-102.

Lau, R.H. and Dolittle, W.F. 1979. Covalently closed circular DNAs in closely related unicellular cyanobacteria. **J. Bacteriol. 137**:648-652.

Lau, R.H., Sapienza, C. and Doolittle, W.F. 1980. Cyanobacterial plasmids: Their widespread occurence and the regions of homology between plasmids in the same and different species. **Mol. Gen. Genet. 178**:203-211.

Lea, P.J., K.W. Joy, J.L. Ramos and M.G. Guerrero. 1984. The action of 2-amino-4- (methyl phosphinyl) butanoil acid (phosphinothrincin) and its 2-oxo derivative on the metabolism of plants. **Phytochem., 23**: 1-5.

Leon, C., S. Kumazawa and A. Mitsoi. 1986. Cynic appearance of aerobic nitrogenase activity during synchronous growth of unicellular cyanobacteria. **Curr. Microbiol., 13**: 149-143.

Liengen, T., and R.A. Olsen. 1997. Nitrogen fixation by free living cyanobacteria from different coastal sites in a high artic tundra. Spitrbergen. **Artic and Alpine Research,** 29(4): 470 - 477.

Lowry, O.H., N.J. Rosebrough, A.C. Larr and R.I. Randall. 1951. Protein measurement with folin phenol reagent. **J. Biol. Chem., 93**: 265 - 275.

Mac Coll, R. and Gurard-Friar.1987. Phycobiliproteins, CRC Press, Inc., Boca Raton, Florida, USA, p.

Madhusoodanan, P.V. and T.K. Dominic. 1995. On the acid tolerance of certain strains of cyanobacteria from Kerala. **Natl. Symp. Algal Biofertilizer**, Tamilnadu Agri. Univ., Coimbatore.

Malcom Devine, O. Stephen Duke and Carl Fedke. 1993. **Physiology of herbicide action.** Prentice Hall, Inc. New Jersey. pp. 99.

Maniatis, T., E.F. Frisch and J. Sambrook. 1982. **Molecular cloning; A laboratory manual**. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Margheri, C. and L. Tomaselli. 1995. Fatty acid based classification of cyanobacteria. **Phytochemistry, 96**(1): 102 - 107.

Markov, S.A., D.G. Polesskaya and A.A. Kransnovski. 1992. Reversible photostimulation of N_2 fixation and H_2 evolution in the BGA. *Anabaena variabilis*. Soviet. **Plant. Physiol., 38**(5): 653-658.

Mazur, B.J., D. Rice and R. Haselkorn. 1980. Identification of blue-green algal nitrogen fixation genes by using heterologous DNA hybridization probes. **Proc. Natl. Acad. Sci. U.S.A., 77**:186-190.

McDaniel, H.R., J.B. Middle Brook and R.O. Bowman. 1962. Isolation of pure cultures of algae from contaminated cultures. **Appl. Microbiol., 10**:223.

Meeks, J.C., N. Steinberg, C.M. Joseph, C.S. Enderlin, P.A. Jorgenson and G.A. Peters. 1985. Assimilation of exogenous and dinitrogen derived $^{13}NH_4^+$ by *Anabaena azollae* separated from *Azolla caroliniana* wild. **Arch. Microbiol., 142**:229-233.

Mehta, R.S. and K. Hauxby. 1977. Action of herbicides on blue green algae - ultrastructural observation on photosynthetic lamellae. **Misc. Ser., Bot. Soc. Amer., Pub. 154**. p. 51.

Merina Prem Kumari, S. and S.Kannaiyan. 1994. Production of growth promoting substances by *Anabaena azollae*. In : **National Seminar on *Azolla* and Algal Biofertilizer for Rice** (ed.) S.Kannaiyan, TNAU, Coimbatore, India, p.5.

Millineaux, C.W. 1994. Excitation energy transfer from phycobilisomes to photosystem I in a cyanobacterial mutant lacking photosystem II. **Biochem. Biophys. Acta., 1184**: 71 - 77.

Moore, S. and D.A. Dorward. 1968. Accumulation and metabolism of pesticides by algae. **J. Phycol., 4**:7-11.

Nayak, H. D. Sahu and S.P. Adhikary. 1996. Blue green algae of rice fields of Orissa state II. Growth and nitrogen fixing potential. **Phykos, 35**(1and 2): 111-118.

Newton, J.W. and D.D. Tyler. 1987. Involvement of photosystem II in the ammonia metabolism of a heterotrophic cyanobacterium. **Biochem. Biophys. Acta., 891**(1): 49-55.

Nicholson, M.L., Gaasenbeek, M. and Laudenbach, D.E. 1995. Two enzymes together capable of cysteine biosynthesis are encoded on a cyanobacterial plasmid. **Mol. Gen. Genet. 247**:351-354.

Okada, A. and M. Yamaguchi. 1955. Nitrogen fixing microorganisms in paddy soils. I. Characteristics of nitrogen fixation in paddy soils. **Soil and Plant Food.1**:102-104.

Pachpande, R.R. 1990. Rate of algal biofertilizer for increasing yield of irrigated plantation crops. In: **National Symposium on Cyanobacterial Nitrogen Fixation** (ed.) B.D. Kaushik. Indian Agri., Res. Inst., New Delhi. pp:27-30.

Padhy, R.N. 1985. Cyanobacteria and pesticides. **Residue Rev., 95**: 1 - 44.

Pakrasi, I.B. and L.A. Sherman. 1984. Highly active oxygen evolving photosystem II preparation from the cyanobacterium *Anacystis nidulans*. **Plant Physiol., 74**: 742 - 745.

Palinska, K.A., W. Liesack, E. Rhie and W.E. Krumbein. 1996. Phenotype variability of identical genotypes the need for a combined approach in cyanobacterial taxonomy demonstrated on **Merimopedia** like isolates. **Arch. Microbiol., 105**: 224 - 233.

Pandey, K.D. and A.K. Kashyap. 1986. Differential sensitivity of three cyanobacteria to the rice field herbicide machete. **J. Basic. Microbiol., 26**: 421 - 428.

Panigrahy, K.C. 1984. **Physiological and genetical effect of pesticides on blue green algae: effect of carbamate pesticides**. Ph.D Thesis. Behrampur University, Behrampur.

Panse, V.G. and P.V. Sukhatme. 1985. **Statistical methods for Agricultural workers**. Indian Council Agri. Res., New Delhi.

Paroda, R.S. 1999. For a food secure future. In:**The Hindu Survey of Indian Agriculture** (ed.) N. Ravi, National Press, Chennai, pp. 17-23.

Parry, A.D. and R. Horgan. 1991. Carotenoids and absisic acid biosynthesis in higher plants. **Plant. Physiol., 82**: 320 - 326.

Peters, G.A., T.B. Ray, B.C. Mayne and R.E. Toia. 1980. *Azolla - Anabaena* association morphological and physiological studies. In : **Nitrogen fixation** (eds.) W.E. Newton and W.H. Orme Johnson, Univ. Park Press, Baltimore, pp.293-309.

Pillay, A.R. and Y.T. Tchan. 1972. Study of soil algae. Adsorption of herbicides in soil and prediction of their rate of application by algal method. **Plant and Soil, 36**: 571 - 575.

Pinevich, K. 1986. Effect of nitrogen starvation on the photosynthesizing apparatus of *Anabaena variabilis* mutant defective for the system of nitrogen fixation. **Microbiologia, 55**(3):418-424.

Prasperi, C., L. Boluda, C. Luna and E.F. Valiente. 1992. Environmental factors affecting *in vitro* nitrogenase activity of cyanobacteria isolated from rice fields. **J. Appl. Phycol., 4**(3):197-204.

Rai, A.N., P. Rowell and W.D. P. Stewart. 1984. Evidence for an ammonium transport system in a free-living and symbiotic cyanobacteria. **Arch. Microbiol., 137**: 241-246.

Ramos, J.L., M.G. Gurrero and M. Losada. 1981. Photoproduction of ammonia from dinitrogen by whole cells of blue green algae. In: **Proceedings of the Fifth International congress on photosynthesis. Vol. 6** (ed). G. Akoyunoglou, Balaban International Science Services, Philadelphia, pp. 707-717.

Rather, M.D. 1994. Herbicide effect on growth and nitrogen fixation of *Nostoc muscorum* In: **Advances in Plant Science Research**, Vol II. (ed.) K.C. Sahni. International Book Distributors, Dehradun. pp.201-218.

Rathore, D.S., A. Kumar and H.D. Kumar, 1993. Lipid content and fatty acid composition in N_2 fixing cyanobacterium. *Anabaena dolionum* as affected by molybdenum deficiency. **World J. Microbiol. Biotechnol.,** 9(5): 508-510.

Reigh., S. and P. Boger. 1989. Regulation of nitrogenase activity in *Anabaena variabilis* by modification of the Fe-protein. **FEMS Microbiol. J., 58**: 18-86.

Reigh, S., H. Almon and P. Boger. 1987. Comparing short-term effects of ammonia and methylamine on nitrogenae activity in *Anabaena variabilis* (ATCC 29413). **Z. Naturforsch., 42C**: 902-90.

Rippka, R. and J.B. Waterburry. 1977. The synthesis of nitrogenase by non heterocystous cyanobacteria. **FEMS Microbiol. Lett., 2**:83-86.

Roberts, T.M. and K.E. Koths. 1976. The blue-green alga *Agmenellum quadruplicatum* contains covalently closed DNA circles. **Cell, 9**:553-557.

Roger, P.A. and S.A. Kulasooriya. 1980. **Blue-green algae and rice**. The International Rice Research Institue, Los Banos, Laguna, Philippines, pp. 112.

Roger, R., C. Harms, M. Hebles and L.H. Grime. 1994. Cyclic variations of photosynthetic activity under nitrogen fixing conditions in *Synecococcus* RF - 1. **Arch. Microbiol., 162**(1 and 2): 80 - 84.

Roychoudhury, P and B.D. Kaushik. 1986. Response of cyanobacterial growth and nitrogen fixation to herbicides. **Phykos, 25**: 36 - 43.

Rucker, J., J.G. Kohl and K. Kaiser. 1995. Response of carotenoids and chlorophylls to variations of growth limiting factors in three filamentous blue green algae. **Archiv Fuer Hydrobiologie Supplement band, 108** (0): 51-65.

Ryther, J.H. and M.D. Hanisak. 1982. Anaerobic digestion and nutrient recycling of small benthic or floating sea weeds. In : **Energy from biomass and wastes** V. (ed.) D.L.Klass, Institute of gas technology, Chicago, pp.384-410.

Sankaram, A. 1971. Work done on blue green algal in relation to agroculture. **New Delhi Bull. No.27**, pp 28.

Santra, S.C. 1993. **Biology of Rice Fields Blue Green Algae**, Daya Publishing House, Delhi. pp. 152.

Selvamani, S. and S. Sankaran. 1993. Soil microbial population as affected by herbicides. **Madras Agric. J., 80**(7):397-399.

Shi, D.J., and D.O. Hall. 1988. The *Azolla-Anabaena* association: Historical perspectives, symbiosis and energy metabolism. **Bot. Rev., 54**:353-386.

Shivaram, S. and K. Shivappa Shetty. 1988. Studies on the effect of pesticides on the growth and N_2 fixation by blue green algae. **Mysore J. Agric. Sci., 21**:222-225.

Show, Jr. I.T. 1981. Marine plants. In : **CRC Handbook of biosolar resources**. Vol.II. Resource materials, (eds.) O.R.Zaborsky, T.A. McClure, E .S. Lipinsky, CRC Press, Boca Raton, FL, USA), pp.471-498.

Siefermann-Harms, D. 1985. Carotenoids in photosynthesis I. Location in photosynthetic membranes and light harvesting function. **Biochem. Biophys. Acta., 811**: 325 - 355.

Siefermann-Harms, D. 1987. The light harvesting and protective functions of carotenoids in photosynthetic membranes. **Plant Physiol., 69**: 561 - 568.

Singh, P.K. 1972. Effect of pesticides on blue-green algae. **Proc. 13 Ann. Conf. Amoc. Microbiol. India**. 56.

Singh, P.K. 1974. Algicidal effect of 2,4-dichlorophenoxy acetic acid on blue green alga **Cylinderospermum** sp. **Arch. Microbiol., 97**: 69 - 72.

Singh, R.N. 1961. **Role of blue green algae in nitrogen economy of Indian agriculture**. Indian Council Agri. Res. New Delhi, p. 175.

Singh, H.N. and A. Vaishampayan. 1978. Biological effect of rice field herbicide machete on various strains of the nitrogen fixing blue green alga *Nostoc muscorum*. **Env. Exp. Bot., 18**: 87 - 94.

Singh, L.J. and D.N. Tiwari. 1988. Effect of selected rice field herbicides on photosynthesis, respiration and nitrogen assimilating enzyme systems of paddy soils diazotrophic cyanobacteria. **Pestic. Biochem. Physiol., 31**: 120 - 128.

Singh, D.T., D.R. Modi and H.N. Singh. 1986. Evidence for glutamine synthetase and methyl ammonium (ammonium) transport system as two distinct targets of methionine sulfoximine inhibition action in the cyanobacterium *Anabaena dolionum*, **FEMS Microbiol. Lett., 37**:95-98.

Singh, S., B.B. Singh and P.S. Bisen. 1995. Role of ammonium assimilation and urea inhibition of nitrogenase activity in *Nostoc*. **Indian J. Microbiol., 18**(2): 128-130.

Singh, V.P., B.D. Singh, R.B.Singh, B.Dhar, R.M.Singh and J.S. Srivatsava. 1978. Effect of herbicide alachlor on growth and nitrogen fixation in cyanobacteria and rhizobia. **I. J. Expt. Biol., 16**:1325-1327.

Solorzano, L. 1969. Determination of ammonia in natural water by the phenol hypochlorite method. **Limol. Oceanogr., 14**: 799 - 801.

Stangel, P.J. 1984. World nitrogen situation trends, outlooks and requirement. In : Nitrogen in crop production (ed.) R.D. Hauck, Am. Soc. Agron. Madison, Wisconsin, USA, 23-54.

Stewart, A.C. 1988. Molecular biology of photosynthetic reaction. In: **Biochemistry of the algae and cyanobacteria**. Clarendon Press, Oxford, pp. 105 - 117.

Stewart, W.D.P. and H.W. Pearson. 1960. Effect of aerobic and anaerobic conditions on growth and metabolism of blue green algae. **Proc. R. Soc. London. Ser.B., 175**: 293-311.

Stewart, W.D.B. and P. Rowell. 1975. Effects of L-methionine DL sulphoxamine on the assimilation of fixed NH_3 and acetylene reduction and heterocyst production in *Anabaena cylindrica*. **Biochem. Phys. Commun., 65**: 846 - 856.

Stewart, W.D.P., P. Rowell, J.K. Ladha and M.J.A.M. Sampaio. 1979. Blue green algae (cyanobacteria) some aspects related to their role as sources of fixed nitrogen in paddy soils. Proc. Nitrogen Rice Symp., Inte. Rice Res. Inst., Manila, pp.263-283.

Stratton, G.W. 1984. Effects of the herbicide atrazine and its degradation products alone and in combination on photo trophic microorganisms. **Arch. Environ. Contam. Toxicol., 13**: 35 - 42.

Subramaniam, G. and S. Shanmugasundaram. 1986. Influence of herbicide 2,4-D on nitrogen fixation and ammonia excretion by the cyanobacterium *Anabaena*. **Proc. Indian Natl. Sci. Acad.**, B52, pp.308-312.

Subramanian, G. and S. Shanmugasundaram. 1987. The influence of nitrate, pH and light duration on the growth of *Anabaena*. **Phykos, 26**: 159-164.

Suguna Rani, K. 1997. **Studies on the growth, nitrogen fixing acitivity, pigment contents and ammonia excretion by the saline tolerant cyanobacteria**. M.Sc.(Agri) Thesis, Tamil Nadu Agricultural Univ. Coimbatore.

Swain, N.B. Rath, S.P. Adhikary. 1994. Growth response of the cyanobacterium *Microcystis aeroginosa* to herbicides and pesticides. **J. Basic Microbiol., 34**(3): 197-204.

Talling, J.F. and T. Driver. 1961. Some problems in the estimation of chlorophyll-a in phytoplankton. In: Primary productivity measurements in marine and fresh water. 10th Pacific Sci. Long Div. Tech. Inform. US Atomic Energy Commission, pp. 142 - 140.

Tamilselvam, B. 1998. **Selection of fast growing and higher nitrogen fixing acid tolerant cyanobacterial cultures and their utility as biofertilizer for rice**. M.Sc(Agri) Thesis, Tamil Nadu Agri. Univ. Coimbatore.

Thomas, J. and S.K. Apte. 1984. Sodium requirement and metabolism in nitrogen fixing cyanobacteria. **J. Biosci., 6**:771-794.

Tiwari, D.N., A. Kumar and A.K. Mishra. 1991. Use of cyanobacterial diazotrophic technology in rice agriculture, **Appl. Biochem. Biotechnol., 28-29**: 87-396.

Tsygankov, A.A., C. Van Ni and I.N. Gogotov. 1992. *Anabaena variabilis* in continuous culture growth and adaptation potential of its nitrogenase system. **Microbiol., 60**(3): 591-595.

Vaishampayan, A. 1985. Mutagenic activity of alachlor butachlor and carbaryl to a nitrogen fixing cyanobacterium *Nostoc muscorum*. **J. Agric. Sci., 104**: 571 - 576.

Vance, B.D. and W. Drummond. 1969. Biological concentration of pesticides by algae. **J. Amer. Water. Works. Assoc., 61**: 360-365.

Veena Nagpal and S.K. Goyal. 1992. Growth responses of cyanobacteria to herbicides. **Acta. Bot. Ind., 20**: 173 - 176.

Venkataraman, G.S. 1972. **Algal Biofertilizer and Rice cultivation** Today and Tomorrow Printers, New Delhi, p. 75.

Venkataraman, G.S. 1979. Algal inoculation in rice fields. In : Nitrogen and Rice. Inte. Rice Res. Inst., Los Banos, Philippines, pp.311-321.

Venkataraman, G.S. 1981. Blue green algae : a possible remedy to nitrogen scarcity. **Curr. Sci., 50**: 253 - 256.

Venkataraman, G.S. and B. Rajyalakshmi. 1971. Tolerance of blue green algae to pesticides. **Curr. Sci., 40**: 143 - 144.

Venkataraman, G.S. and B. Rajyalakshmi. 1972. Relative tolerance of nitrogen fixing blue green algal to pesticides. **Curr. Sci., 40**: 143 - 145.

Venkataraman, G.S. and B. Rajyalakshmi. 1974. **Tolerance of blue green algae to pesticides. Curr. Sci., 6**: 143 - 144.

Venkateshwaralu, K. 1993. Pesticides interactions with cyanobacteria in soil and pure cultures. In: **Soil Biochemistry** Vol. 8 (eds.). J.M. Bollag and G. Slotzky, Marcel Dekkar, New York, pp. 137-179.

Waksman, S.A. 1962. **Soil Microbiology**, John Wiley and Sons, Inc, Newyork, USA,p.356.

Ward, D.M., C.M.Santegoeds, S.M.Nold, N.B.Rensing, M.T.Ferris, and M.M.Bateson. 1997. Biodiversity within hot spring microbial mat communities. Molecular monitoring of enrichment cultures. **In: Microbial physiology and gene regulation emerging principles and applications**. (ed.) A.H.Southamer, **Antonie Van leeuwenhoek 71**(1-2): 143 - 147.

Windhovel, V., B. Geiges, B. Sandman and P. Boger. 1994. Expression of Erwina uredovora phytoene desaturase in Synecococcus PCC 7942 leading to resistance against a bleaching herbicide. **Plant Physiology, 104**(1): 119-125.

Winterman, J.F.G.M. and A. Demotes. 1965. Spectrophotometric characteristic chlorphylls a and b and their phenophytins in ethanol. **Biochem. Biophys. Acta., 109**: 448 - 453.

Wolk, C.P., J. Thomas, P.W. Shaffer, S.M. Austin and A. Galonski. 1976. Pathway of nitrogen metabolism after fixation of ^{13}N- labelled N_2 gas in the cyanobacterium *Anabaena cylindrica*. **J. Biol. Chem., 251**: 2027-2034.

Wright, S.J.L. 1978. Interactions of pesticides with microalgae. In: **Pesticide microbiology** (eds.) I.R. Hill and S.J.L. Wright. Academic Press, London, pp.157-164.

Yanni, Y.G. 1992. The effect of cyanobacteria and *Azolla* on the performance of rice under different levels of fertilizer nitrogen. **World J. Microbiol. Biotechnol., 8**: 132-136.

Yoshida, T. and R.R. Ancaja,. 1973. Nitrogen fixation activity in upland and flooded rice. **Soil Sci. Soc. Amer. Proceedings, 37**:42-46.

Zagar, M.Y. and Dar. G.H. 1990. Effect of benthiocarb and butachlor on growth and nitrogen fixation by cyanobacteria. **Bull. Environ. Contd. Toxicol., 45**: 232 - 234.