CHARACTERISATION OF THE CYANOBACTERIA *SCYTONEMA CINCINNATUM* THURET EX BORN. ET FLAH. (SCYTONEMATACEAE) AND *WESTIELLOPSIS PROLIFICA* JANET (STIGONEMATACEAE) OF ACIDIC SOILS OF KERALA

Thesis submitted to the University of Calicut
in partial fulfilment of the requirements
for the degree of
Doctor of Philosophy

By

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CERTIFICATE

Certified that the thesis entitled **Characterisation of the cyanobacteria *Scytonema cincinnatum* Thuret ex Born. et Flah. (Scytonemataceae) and *Westiellopsis prolifica* Janet (Stigonemataceae) of acidic soils of Kerala** submitted by **Ms. Shamina M.** for the degree of **Doctor of Philosophy** in Botany of the University of Calicut is a bonafide record work done by her in this Department under my supervision. This has not previously been formed the basis for the award of any degree / diploma.

Calicut University,
30th January 2006.

Dr. P.V. Madhusoodanan

DECLARATION

I hereby declare that the thesis entitled **Characterisation of the cyanobacteria *Scytonema cincinnatum* Thuret ex Born. et Flah. (Scytonemataceae) and *Westiellopsis prolifica* Janet (Stigonemataceae) of acidic soils of Kerala** submitted for the degree of Doctor of Philosophy in Botany of the University of Calicut is an original research work done by me and no part of this has been submitted for the award of any other degree or diploma.

Calicut University,
30th January 2006.

Shamina M.

Dedicated
to my parents and to my teachers

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CONTENTS

ABBREVIATIONS AND SYMBOLS

ADP	-	adenosine diphosphate
APC	-	allophycocyanin
AR	-	Analytical Reagent
ARA	-	acetylene reduction assay
ATP	-	adenosine triphosphate
BDH	-	British Drug House
BGA	-	Blue Green Algae
BNF	-	Biological Nitrogen Fixation
C_2H_4	-	ethylene
$CaCl_2$	-	calcium chloride
cm	-	centimetre
CO_2	-	carbondioxide
$Co(NO_3)_2$	-	cobalt nitrate
$CuSO_4$	-	copper sulphate
D.D.W.	-	double distilled water
e.g.	-	*exampli gratia*
EDTA	-	ethylene diamine tetra acetic acid
Eh	-	electrical conductivity
et al.	-	*et alia*, and others
etc.	-	*et cetera*
$FeCl_3$	-	ferric chloride
Fig.	-	Figure
g	-	gram
$g.l^{-1}$	-	gram per litre
h	-	hour

H_2O	-	water
H_2SO_4	-	sulphuric acid
H_3BO_3	-	boric acid
ha	-	hectare
HCl	-	hydrochloric acid
HCO_3	-	carbonate
i.e.	-	*id est*, that is
IARI	-	Indian Agricultural Research Institute
lbs	-	pound
In situ	-	in nature
ln	-	natural logarithm
IRRI	-	International Rice Research Institute
k	-	division per day
K^+	-	potassium ion
K_2HPO_4	-	di potassium hydrogen phosphate
K_2SO_4	-	potassium sulphate
k_e	-	growth constant
kg ha^{-1}	-	kilogram per hectare
kg cm^{-2}	-	kilogram per centimetre square
KNO_3	-	potassium nitrate
KOH	-	potassium hydroxide
kpa	-	kilo pascal
lux	-	luminous intensity
l	-	litre
$MgSO_4$	-	magnesium sulphate
$MnCl_2$	-	manganese chloride
M ha	-	million hectare

M	-	Molar
mg	-	milli gram
ml	-	milli litre
mM	-	milli molar
MT	-	metric tonnes
n mol ml^{-1}	-	nano mole per millilitre
N	-	normal
N_2	-	molecular nitrogen
Na^+	-	sodium ion
Na_2CO_3	-	sodium carbonate
NaCl	-	sodium chloride
NaOH	-	sodium hydroxide
NADPH	-	nicotinamide adenine dinucleotide phosphate
$NaNO_3$	-	sodium nitrate
Na_2MoO_4	-	sodium molybdate
NH_4^+	-	ammonium ion
NH_4Cl	-	ammonium chloride
$(NH_4)_2SO_4$	-	ammonium sulphate
NO_3	-	nitrate
NPK	-	Nitrogen, Phosphorus, Potassium
O_2	-	oxygen
^oC	-	degree Celsius
P_{700}	-	photosystem 700
PBS	-	phycobilisomes
PC	-	phycocyanin
PE	-	phycoerythrin
PGR	-	Plant growth regulators

pg	-	picogram
pH	-	negative logarithm of hydrogen ion concentration
Pi	-	inorganic phosphate
ppm	-	parts per million
PS I	-	photosystem I
PS II	-	photosystem II
Qp	-	phosphorus quota
q/ha	-	quintal per hectare
rpm	-	revolutions per minute
S.D.	-	standard deviation
sp.	-	species
UK	-	United Kingdom
USA	-	United States of America
UV	-	Ultra violet
v/v	-	volume per volume
viz.	-	*Vide licet*, namely
w/v	-	weight per volume
$ZnSO_4$	-	zinc sulphate
%	-	per cent
µm	-	micrometre
µ mol	-	micro mole
µl	-	microlitre
$\mu g.ml^{-1}$	-	microgram per millilitre
&	-	and
±	-	plus or minus
<	-	less than
>	-	greater than

Introduction

1. INTRODUCTION

Cereals form the staple food of majority of population of the Indian subcontinent. Among cereals, rice is one of the indispensable staple food crops of India and it occupies 43 million hectare land with annual production of 81 million tonnes *i.e.*, about 43 per cent of the nation's total food grain production. Our agricultural production has witnessed multifold increase during the past five decades. There is an urgent need to boost productivity per unit area per unit time to meet the food demand of ever increasing human population. India has already attained one billion population and it has been estimated that by 2025 the population will reach 1.3 billion with a potential food grain demand of around 315 million tonnes (Venkataramani, 2000). The introduction of high yielding varieties during the Green Revolution era has been the major break through in Indian agriculture in increasing the food grain production. These high yielding varieties are highly responsive to inorganic fertilizers. The rice crop requires a good nutrient source for better production (Mishra *et al.*, 2003). Thus a balanced use of these inorganic fertilizers has become an essential part of the crop production. But these fertilizers pollute the environment through the process of denitrification and volatilization and soil water through leaching. Only 50% of available nitrogen is being used and rest 50% goes as wasted, thus posing a major threat to sustained soil health and crop productivity (Panwar *et al.*, 2001). The present world production of nitrogen fertilizer is roughly 60-65 million MT and farmers in developing countries spend over 6.5-7.0 billion dollars each year

for nitrogen fertilizer alone. In India, the current fertilizer consumption is roughly 18-19 million tonnes with average use of 90 kg nitrogen fertilizer per hectare (Upasana *et al.*, 2001). The recent energy crisis and consequent price hike of fertilizers coupled with the low purchasing power of the farming community has become a limiting factor for crop production (Choudhary and Thakur, 2003). In this context, an alternative method of ecofriendly use such as application of biofertilizers, has gained great priority in modern agriculture.

Biofertilizers are living fertilizers composed of microorganisms which are capable to fix atmospheric nitrogen. The term 'biofertilizer' denote all the inputs of biological origin for plant growth (Subba Rao, 1982). Biofertilizers are efficient nitrogen fixing, phosphate solubilizing or cellulose decomposing microorganisms. They enhance availability of nutrients to plants on application to seed or soil and offer an ecofriendly, economically viable and socially acceptable means of reducing external input of chemical fertilizers. Biofertilizer is a boon for farmers and a need of time because it aids in increasing soil fertility. Biofertilizers mixed with compost and manure help in their survival and better environment for their activities. It improves soil quality, soil texture, structure and productivity by enhancing the granulation, porosity, water holding capacity, nutrient supply rate and activity of soil biota and also proliferate useful soil microorganisms (Singhal *et al.*, 2003).

Cyanobacteria (Blue-green algae) are the largest, most diverse and most widely distributed groups of photosynthetic N_2 fixing prokaryotes

(Stanier and Cohen-Bazire, 1977). Cyanobacterial inoculation in rice crop is now a recommended management practice. Venkataraman (1961) coined the term algalization to denote the process of application of blue-green algae culture in the field as biofertilizer. India has been the pioneer in the field of cyanobacterial biofertilizer technology and has generated valuable information on practical utilization of this important biological input in crop production. The judicious use of these cyanobacteria could provide to our country's entire rice acreage as much nitrogen as obtained from 15 to 17 lakh tonnes of urea (Venkataraman, 1988). Even though cyanobacterial nitrogen fixation promises a lot in rice field ecosystem, the concept of using this self supporting diazotroph as a potential biofertilizer is not yet fully exploited (Pabbi *et al.*, 2000).

The mass production technologies to produce these biofertilizers have undergone several modifications to increase productivity and improve quality. Cyanobacterial biofertilizers have become very popular among rice farmers in many parts of our country. The major constraint experienced by many researchers and extension workers involved in this technology, is the identification of effective strains and production of quality inoculum.

The availability of nitrogen in an utilizable form is a prerequisite for plant growth. In natural environments a delicate balance is established between the rate of plant growth and the supply of available soil nitrogen (Date, 1973). The amount of nitrogen available in the soil is controlled by the rate of accumulation of nitrogen through nitrogen fixation and the

processes of ammonification and nitrification of organic nitrogen and by the rate of loss of nitrogen via leaching or denitrification. Under conditions of intensive agriculture, this delicate balance is disturbed because organic nitrogen in the form of plant material is continually removed from the land and not directly recycled. The principal factor limiting plant growth now becomes the rate of addition of fixed nitrogen to the ecosystem. For this reason, intensive agricultural systems have become dependent upon the application of nitrogenous fertilizer, at one time a convenient and inexpensive source of fixed nitrogen (Hardy and Havelka, 1975). The yield potential of many agronomically important crop plants is intimately linked to the availability of nitrogen fertilizers (Hardy and Havelka, 1975). The production of nitrogenous fertilizers is an energy intensive process. Natural gas equivalent to approximately $3x10^8$ barrels of oil is consumed annually for the synthesis of anhydrous ammonia by the Haber – Bosh process for use in nitrogen production. The biological nitrogen fixation contributes 70% of the total nitrogen fixed world wide ($175x\ 10^6$ metric tonnes per year), whereas the industrial production of nitrogenous fertilizers accounts for only 15% (Burns and Hardy, 1975). Currently the production of cereal crops is greatly dependent upon the application of fertilizer nitrogen and thus attention has been focused on relieving this uneconomical and unhealthy dependence.

1.1. Objectives of the present study

(1) Comparative study on two species of commonly occurring rice field cyanobacteria, one belongs to the order Nostocales showing false

branching (*Scytonema cincinnatum*) and other belongs to the order Stigonematales showing true branching (*Westiellopsis prolifica*) on growth, ammonia excretion and other biochemical parameters.

(2) Studying the growth, protein content, pigment concentrations and ammonia excretion of cyanobateria on different culture media in laboratory conditions.

(3) To study the growth, protein content, pigment concentrations and ammonia excretion at various pH levels and NaCl concentrations.

(4) To study the mixotrophic, photoheterotrophic and chemoheterotrophic growth capability of cyanobacteria.

(5) To study the influence of various agrochemicals, organic supplements and PGR like carbofuran, neemcake and 2,4-D on the growth of cyanobacteria.

(6) To study the response of cyanobacteria to organic and inorganic forms of fertilizers such as nitrogen, phosphorous and potassium.

(7) To study immobilization of cyanobacteria for long term storage.

(8) To assess the performance of cyanobacteria as a biofertilizer for the growth and yield of rice.

Review of Literature

2. REVIEW OF LITERATURE

2.1. PHYLOGENY OF CYANOBACTERIA

More than three billion years ago the advent of primordial cyanobacteria marked a decisive turning point in the evolution of life on our planet. Starting to introduce free oxygen into a previously anoxygenic biosphere, cyanobacterial plant type photosynthetic machinery allowed a transmembrane charge separation to occur (Skulachev, 1994). The Proterozoic Era, a vast tract lasting almost 2 billion years, is called as 'Age of cyanobacteria', because it is thought that the earth's biota was dominated by these organisms. During this period, cyanobacteria gradually increased the oxygen content of the atmosphere and thus paved the way for obligatory aerobic organisms the eukaryotes in particular but also aerobic bacteria. During the Proterozoic radiation, the cyanobacterial species with heterocysts evolved, showing that atmospheric O_2 concentration had risen to a level sufficiently high to poison the nitrogenase enzyme, which catalyzes the fixation of atmospheric N_2. The non heterocystous cyanobacteria alive today are capable of N_2 fixation are apparently relicts from the pre-oxygen era (Hoffmann, 1985; Schopf, 1978; Schopf and Walter, 1982; Tappan, 1980; Vidal, 1984). The cyanobacteria acquired photosystem II and thereby the capacity for oxygenic photosynthesis, and it was only after this critical evolutionary innovation that the development of an oxygen rich atmosphere and sea are possible (Waterbury and Rippka, 1989). Cyanobacteria form a

link between prokaryotes and eukaryotes and have tremendous capacity to adopt to various extreme environmental conditions like high temperature, cold, marine and toxic environments (Bisen *et al.*, 1993). These oxyphotosynthetic bacteria may be considered as precursors of the present day chloroplast plants and rhodophytes and they share the common features of genomic structures and functions with bacteria (Ris and Singh, 1961; Stanier and Van Niel, 1962; Lang, 1968; Stanier, 1977) but differ from them in having oxygenic mode of photosynthesis (Haselkorn, 1978). The apex fossil deposits in Western Australia contains a variety of multicellular filaments which resemble certain modern cyanobacteria such as *Oscillatoria* and some modern non photosynthetic bacteria (Schopf, 1993). Many filamentous structures found in proterozoic rocks are strikingly like modern filaments. Derivatives of 2-methyl bacteriohopanepolyols, which occurs in many modern cyanobacteria have been found in organic rich sediments as old as 2500 million years. Some of the reasons for the success of the cyanobacteria in modern habitats can be related to their long evolutionary history (Summons *et al.*, 1999). Cyanobacteria are the oldest group of microorganisms and they have been found on the earth for more than 3.5 billion years. During this process, they evolved several adaptive mechanisms, both structural and functional as strategies for survival and growth (Mann, 2000; Bhaya *et al.*, 2000). They added oxygen to the atmosphere and paved the path for evolution of aerobic forms including the aerobic bacteria.

Other evidence for ancient oxygenic photosynthesis comes from 3.5 billion years old calcareous structures known as stromatolites, found in sheltered parts of Shark Bay, Western Australia. They are formed in permanently submerged sites where upward growing mats of cyanobacteria trap sediments and laminations are formed through periodic variations in cyanobacterial activity. Calcium carbonate precipitation with in gelatinous sheaths of the cyanobacteria also contribute to the formation of stromatolites (Monty, 1977). Stromatolites are widely distributed in the sedimentary rocks of Precambrian age through out the world and considered as organically preserved microfossils of cyanobacteria. These microfossils include both unicellular, coccoid and multicellular filamentous forms (Logan *et al.*, 1964; Brock, 1973a).

2.2. OCCURRENCE AND DISTRIBUTION

Cyanobacteria are oxygen evolving, nitrogen fixing prokaryotes occur in every conceivable habitats but abundantly in waterlogged rice fields in several rice growing countries (Gopalaswamy, 2001). They are ubiquitous in nature and next to bacteria in their distribution and constitute a morphologically diverse and widely distributed group of photosynthetic prokaryotes. They show greatest abundance in tropical regions than temperate soils. The cyanobacteria constitute 75 per cent of the total algal flora in Indian paddy fields (Pandey, 1965). They are called as primary colonizers since they are able to colonize inhospitable ecosystems like cold lava from volcanic eruptions (Henriksson and Henriksson, 1978), thermal

springs (Brock, 1967; Castenholz, 1969), icelands (Broady, 1982; Gupta and Kashyap, 1996), bare rocks (Krumbein, 1987; Hoffmann, 1989; Tripathy *et al.*, 1997) and salt affected soils. They can grow luxuriantly in fresh water, sea water, salt marshes, moist rocks, tree trunks, moist soils, hot springs, frozen waters and Oasis in Antartica. They live under every type of environment and on every type of substrate. In Yellow Stone Park, colourful algal mats occur in many thermal areas. Their distribution in these heated waters is related to sulfide levels. Where the sulphide concentration are high, the water is acidic and cyanobacteria grow poorly (Brock, 1973a). There are reports on the occurrence of *Phormidium* and *Lyngbya* in the Antartic lakes (Parker *et al.*, 1981; Wharton *et al.*, 1983). On reefs, free living and symbiotic cyanobacteria are important fixers of nitrogen. They form association with almost every group of plants such as green algae, sponges, mosses, ferns, fungi, flowering plants, shrimps, mammals, etc. In majority of the lichens the algal partner is the cyanobacteria (Fogg *et al.*, 1973). These cyanobacteria perform photosynthesis and nitrogen fixation and these photosynthetically fixed carbon and fixed atmospheric nitrogen are translocated from algae to fungus (Fay, 1983).

2.3. CYANOBACTERIA IN RICE ECOSYSTEMS

Nitrogen fixation by cyanobacteria was reported in 1889, shortly after the significance of legume root nodules was found out. Drews (1928) was the first to demonstrate unequivocally that pure cultures of certain cyanobacteria can fix nitrogen. The nitrogenase activity in heterocystous

cyanobacteria was confirmed by using $15N_2$ as tracer (Burris *et al.*, 1943; Stewart *et al.*, 1969).

Cyanobacteria were first reported to be abundant in rice fields by Fritsch (1907) and their agronomic importance due to N_2 fixing ability was first recognized by De (1939). Since then large amount of work has been done to exploit this renewable biological resource as nitrogen input in rice cultivation (Singh, 1961; Subrahmanyan *et al.*, 1965a,b,c; Stewart, 1969, 1973; Fogg *et al.*, 1973; Kaushik *et al.*, 1981, Venkataraman, 1981; Fay and Van Baalan, 1987; Bryant, 1994). Cyanobacteria are especially evident in wetland rice fields which supply 86% of the world requirement of rice (Ladha and Reddy, 1995). These organisms multiply rapidly in the flood water in neutral to alkaline soils and release nitrogen slowly for meeting the nutrient requirement by rice (Tirol *et al.*, 1982; Whitton *et al.*, 1988). The tropical conditions ensure increased incidence of cyanobacteria in the rice field soil which provide a suitable niche for their abundant growth. The luxurient growth of cyanobacteria in the rice fields is due to high humidity, temperature, water, light, and nutrients (Roger and Reynaud, 1979). This could be the reason that cyanobacteria grow in higher abundance in rice field soils than in upland soils as reported in the widely different climatic conditions of the globe (Watanabe and Yamamoto, 1971; Singh, 1985). The aerobic filamentous heterocystous as well as colonial forms represent the common flora of aerable lands and are considered to be important in nitrogen economy of rice cultivation (Kaushik, 2001). During the past few

years, great progress has been made towards elucidating key biological processes such as photosynthesis and N_2 fixation in cyanobacteria. Numerous proteins and genes have been isolated from a wide range of strains and the functions are described (Marsac and Houmard, 1993).

Cyanobacteria are one of the most important groups of organisms of immense ecological importance as they populate many diverse environments. They are the common constituents of the microbial flora of forest and agriculture soils, specially of paddy fields and waters rich in organic matter. The composition and size of cyanobacterial flora in those habitats are controlled by many interacting physical factors like temperature, light, pH, salinity, nutrient concentrations, growth promoting and growth inhibiting substances and selective grazing and chemical factors like carbon content, phosphate content, etc. (Fogg, 1956; Ahlgren, 1970).

2.3.1. Light

Cyanobacteria are principally phototrophic in nature and the light plays an important role in their multiplication. The light has a significant effect on the growth and relative abundance of cyanobacteria in rice fields which was investigated by Quesada *et al.* (1998). Being photoautotrophic, cyanobacteria show definite responses to the quality and quantity of light. The photosynthetic system which is responsible for energy conversion becomes limited at low irradiance, saturated at high irradiance and inhibited at low irradiance. Based on the response of cyanobacteria to light quality, they have been divided into three different groups (Marsac, 1977). Group 1

cyanobacteria can alter phycobilisome (PBS) size and number along with photosystem stoichiometry when grown in different light qualities, but do not dramatically alter the absorbance characteristics of their PBS, through changes in phycobiliprotein composition. Group II cyanobacteria can alter the levels of PE and PC levels. Changes in pigmentation in photosynthetic organisms in response to light quality has been termed chromatic adaptation. When the levels of PE alone as in group II organisms or both PE and PC pigments as in PBS of group III organism are modulated, the process is termed complementary chromatic adaptation. It has been examined in the cyanobacterium *Calothrix* sp. strain PCC 7601, although the phenomenon has been observed in a number of cyanobacteria (Marsac, 1977; Bryant, 1981; 1982). In chromatic adapting cyanobacteria, red light promotes PC synthesis, while green light promotes PE synthesis. The differential expression of PE and PC under different light qualities is suggested to be due to differential transcription of the genes (Mazel *et al.*, 1986; Grossman *et al.*, 1988).

Cyanobacteria are restricted to the photic zone and are usually located in the upper 0.5 cm of the soil (Roger and Kulasooriya, 1980). Light availability for soil algae depends upon the season and latitude, the vertical location of the algae in the photic zone, the cloud cover, the plant canopy and the turbidity of the water. The light intensities reaching the soil may vary from 10 to 110,000 lux (Roger and Reynaud, 1982; Singh, 1984). The dense plant canopy of the dwarf high-yielding varieties play a role in

regulating the availability of light during the cultivation cycle, which in turn, affect the growth and activity of the cyanobacteria in the rice fields. The screening effect of a growing crop canopy appears to cause a rapid decrease in the available light. The canopy of transplanted rice decreased light by 50% when plants were 15 days old, 85% after one month and 95% after two months of growth (Kurosawa,1956). A beneficial effect of the plant canopy shading on the abundance of cyanobacteria was also reported in sugarcane fields, maize fields and grasslands in India (Singh, 1961). In rice fields the light screening effect of crop canopy appears to cause a rapid decrease of light reaching the cyanobacteria but the light tolerance differs between the cyanobacterial species.

The cyanobacteria develop various protective mechanisms against the light intensities such as vertical migration in the water of submerged soil. Preferential growth was more in shaded zone like embankments, under or inside decaying plant material (Kulasooriya *et al.*, 1980) or a few millimeter below the soil surface (Fogg *et al.*, 1973). Migration of cyanobacteria into shaded zones and aggregation provides a self shading effect (Reynaud and Roger, 1978). The deficiency of light may also act as a limiting factor. In Phillipines during the wet season when light was moderate, ARA was higher in basal soil than in planted soil (Watanabe *et al.*, 1977).

In cultures light has a definite role in the N_2 fixation of non-heterocystous forms (Traore *et al.*, 1978) in which there is a temporal separation of photosynthetic O_2 evolution in the light from N_2 fixation in the

dark. In some strains of *Anabaena*, there will be a loss of chlorophyll after 20 days in continuous darkness. They noticed that growth is proportional to the duration of light and the maximum growth could be obtained with continuous illumination (Subramanian and Shanmugasundaram, 1987). In a study by Kaushik (2000) in *Tolypothrix tenius* it was shown that the increase in light intensity from 800 to 5000 lux increases the biomass production and the maximum biomass was synthesized between 4500 and 5000 lux.

2.3.2. Temperatue

Air temperature has a selective role in the growth and multiplication of cyanobacteria. It can grow over wide range of temperatures, but the temperature optima for the growth of cyanobacteria is about 30-35°C. The temperatue is rarely a limiting factor for cyanobacteria in paddy fields, because the range of temperatures permitting the growth of cyanobacteria is longer than that required by rice. However, it influences both algal biomass composition and productivity (Roger and Reynaud, 1979). Low temperature decreases productivity and favours eukaryotic algae whereas, high temperature favours both the phytoplankton productivity and cyanobacteria (Roger and Reynaud, 1977, 1979). Subrahmanyan *et al.* (1965b) reported that in Indian paddy fields the growth of cyanobacteria was inhibited by low temperature. Very high temperature have a deleterious effect on cyanobacterial nitrogen fixation activity (Venkataraman, 1964; Roger and Reynaud, 1977). But the unicellular cyanobacterium *Synechococcus* can tolerate temperatures up to 74°C (Brock, 1967).

High temperature occurring in the surface of the tropical upland soils may have a selective action on the cyanobacterial flora which are more tolerant to high temperature than eukaryotic algae. The dry spores of *Nostoc* sp. could tolerate 2 minutes of 100°C, the wet spores 20 minutes at 60-70°C and the vegetative filaments 10 minutes at 40°C (Chapman and Chapman, 1973). The thylakoid membrane and the embedded redox complexes of the photosynthetic apparatus were found to be the sensitive targets for high temperature stress (Glatz *et al.*, 1999).

2.3.3. pH

Among the soil properties, pH is the most important factor determining the algal flora composition, distribution and its establishment (Roger and Kulasooriya, 1980). In culture media, the optimal pH for cyanobacterial growth seems to range from 7.5 to 10.0 and the lower limit is about 6.5 to 7.0 (Holm-Hansen, 1968; Chapman *et al.*, 1972; Prasad *et al.*, 1978). Under natural conditions cyanobacteria grow preferentially in environments that are neutral to alkaline (Gerloff *et al.*, 1950; Kratz and Myers, 1955; Fogg, 1956; Jurgensen and Davey, 1968; Whitton and Sinclair, 1975; Roger and Reynaud, 1979) which explains that in paddies correlation occurs between water pH and cyanobacterial number (Okuda and Yamaguchi, 1956a,b; Garcia *et al.*, 1973); soil pH and cyanobacterial growth (Okuda and Yamaguchi, 1952) soil pH and N_2 fixing algal mass (Singh, 1974; Roger and Reynaud, 1977).

Acidic soils are therefore one of the stressed environments for these organisms and these are normally absent at pH values below 4 and 5; eukaryotic algae, however flourish under these conditions (Brock, 1973b; Fogg *et al.*, 1973). But there are few exceptional reports on the occurrence of cyanobacteria in high acidic conditions. Dense cyanobacterial bloom has been reported in the soil pH 5.5 in Japan (Matsuguchi and Yoo, 1979) and in acid-bog lands in Swedish soils (Granhall, 1970). There are reports of occurrence of cyanobacteria at pH 3.5 in Kerala (Anand and Hopper, 1987, 1995; Madhusoodanan and Dominic, 1995; Dominic and Madhusoodanan, 1999).

The pH variation in the rice fields occurs due to photosynthetic removal of HCO_3 (Whitton *et al.*, 1988). Light reaching the water layer is the control factor over many physio-chemical characteristics like temperature, pH, alkalinity, CO_2 concentration, since light quality and quantity modify the photosynthetic rate (Mallin and Paerl, 1992). Growth and N_2 fixation by cyanobacteria was affected by change in pH of soil and the medium (Stewart, 1973; Singh, 1974). The beneficial influence of higher pH (8.0 to 10.0) on cyanobacterial growth was demonstrated by the fact that addition of lime increases the cyanobacterial growth and N_2 fixation (Roger and Kulasooriya, 1980). Higher growth and N_2 fixation occurred in the pH range of 7.3-9.8 with optimum N_2 fixation at pH 8.6 in *Aphanothece* sp. (Singh, 1974). The high pH found in Valencian rice fields promoted a high nutritional

status, since alkaline pH can promote more available P and higher rates of nitrification (Leuven *et al.*, 1992).

2.3.4. Salinity

Salinity is one of the most important factors in nature leading to severe crop loss every year and it is an ever increasing problem in agriculture. The area which has become unproductive due to accumulation of salts in the upper profile of soils is about 7-12 M ha in our country. Such soils are grouped under the category of salt affected soils and are classified as saline, saline-sodic and sodic soils. The poor performance of these soils is because of poor soil physical conditions coupled with nutritional disorders related to high exchangeable sodium and pH. The occurrence of cyanobacteria in varying saline situations have drawn much interest in recent years especially on their levels of halotolerance, mechanism of adaptation and role in amelioration of salt affected soils.

An increase in salt concentration represents a combination of two stress conditions for living cells. The reduction in the water potential of the surrounding medium causes the cells to lose water. In contrast to this purely osmotic stress caused by high concentration of non-permeable organic agents, an increase in salinity also means a dramatic increase in inorganic ions especially Na^+ and Cl^-, entering the cell along electrochemical gradients. The physiological basis of salt adaptations is due to the synthesis and accumulation of osmoprotective compounds like carbohydrates (Reed and Stewart, 1988; Anand and Parameswaran, 1990; Kumar and Kaushik,

1994; Padhi *et al.*, 1998); amino acids (Reed *et al.*, 1986; Saxena and Kaushik, 1992; Padhi *et al.*, 1998) and also due to the maintenance of low internal concentrations of inorganic ions by active export mechanisms (Apte and Thomas, 1986; Kaushik and Nager, 1993; Jha and Kaushik, 1988). Recently, it was shown that genes are involved in salt adaptation and these genes can isolate and transferred to cyanobacterial strains of lower salt tolerance to improve their stress tolerance and it also induces the synthesis of special stress proteins (Bhagwat and Apte, 1989; Hagemann *et al.*, 1990).

2.3.5. Akinetes

Cyanobacteria belonging to Nostocaceae, Rivulariaceae and Stigonemataceae differentiate heterocysts and akinetes during different phases of their diazotrophic growth. The akinetes are thick walled, granulated and larger structures than their antecedent vegetative cells and serve the purpose of reproduction and perennation (Fritsch, 1945). Due to their resistance to various unfavourable conditions, the akinetes of cyanobacteria have been equated to bacterial endospores (Nichols and Carr, 1978; Nichols and Adams, 1982).

Akinetes are often produced in large numbers in senescent populations as in ageing water-blooms and ensure survival when the bulk of the population becomes moribund. The precise factors that induce their formation seems to be triggered by phosphate deficiency, whereas in others a lack of energy (as light or carbohydrate) seems to be responsible. In yet other species the differentiation of akinetes is induced by particular organic

compounds that are themselves secreted by akinetes, so that akinete formation is an autocatalytic process (Hoek *et al.*, 1997).

Laboratory experiments have shown that akinetes are capable of surviving extreme environmental conditions, which are not tolerated by vegetative cells. They can endure abnormally high or low temperatures (Whitton, 1987). Akinetes may remain viable for years in anoxic lake sediments. In *Aphanizomenon flos-aquae* of the order Chroococcales, akinetes survive up to 18 years, while in *Anabaena* spp. the survival is up to 64 years (Livingstone and Jaworski, 1980). Upon the return of favourable conditions, as when akinetes are resuspended by turbulence and brought back to the water surface in spring; the increasing light intensity allows germination and subsequently growth (Hoek *et al.*, 1997).

There are various reports on the factors which influence akinete differentiation. In *Anabaena cylindrica* there is a stimulation of akinete differentiation by acetate and calcium glucoronate (Wolk, 1965b), while in *Anabaena doliolum* by glucose (Tyagi, 1974). The addition of sucrose delayed akinete formation in *Nostoc* PCC 7254 (Sutherland *et al.*, 1979). Nitrogen sources such as nitrate nitrogen favoured akinete differentiation in several *Anabaena* spp. while the incorporation of N_2 sources did not affect akinete formation in *Anabaena cylindrica* (Wolk, 1965b), *Nostoc* PCC 7524 (Sutherland *et al.*, 1979) and other *Nostoc* strains (Rippka *et al.*, 1979).

According to Sarma and Garg (1985) inorganic carbon sources such as carbonates and bicarbonates of Na and K and ammonium carbonate

induced akinete differentiation where maximum frequency was not in ammonium carbonate. The incorporation of vitamins like thiamine, biotin, nicotinic acid, ascorbic acid, riboflavin and pyridoxine decreased the frequency of akinetes (Sarma and Swarn Kanta, 1982a, b). The akinetes of certain cyanobacteria are known to germinate too readily in the same medium of their formation (Fay, 1969a; Singh *et al.*,1972; Fogg *et al.*, 1973) or upon transfer to fresh medium (Miller and Lang, 1968; Stulp and Stam, 1982; Sutherland *et al.*, 1985b).

In certain members like *Nostoc* PCC 7524, Sutherland *et al.* (1985a) obtained nearly 100% synchronous germination in fresh medium within 24 hours. According to Van Dok and Hart (1997) low level of cellular phosphorous also triggers akinete production in *Anabaena circinalis* and probably other cyanobacteria as well. They found that akinete differentiation began when the cell phosphorous quota (QP) fell to a critical level of 0.3 to 0.45 pg of phosphorous per cell.

2.3.6. Heterocysts

Heterocysts are differentiated cells and are characterized by thick outer envelope, weak pigmentation and conspicuous refractile granules called polar nodules near the junction of the vegetative cells. Heterocysts are dedicated to the process of N_2 fixation, although some non-heterocystous cyanobacteria can fix atmospheric N_2, if the surrounding environment are anaerobic (Fay, 1983; Fay and Van Baalen, 1987; Gorkom and Donze, 1971; Mattox and Stewart, 1973). It has been reported that the

unicellular forms like *Gleocapsa* (Gallon and Chaplin, 1988; Singh and Tiwari, 1988a,b; Tiwari *et al.*, 1991) and Synechococcus (Mitsui *et al.*, 1987) can also fix atmospheric N_2. Recently, other non-heterocystous forms like *Lyngbya* (Prasanna and Kaushik, 1994) have also been reported to fix nitrogen. Many cyanobacteria produce thick mucilage, which enclose heterocysts and functions as a barrier to oxygen diffusion into cells. This was demonstrated in *Nostoc cordubensis*. Heterocysts coated with mucilage were able to fix nitrogen significantly at higher oxygen concentration than those lacking mucilage (Prosperi, 1994). But the study by Solheim *et al.* (1996) showed that heterocystous cyanobacteria were the most important sources of biologically fixed nitrogen. In laboratory culture the heterocyst differentiation is stimulated only when the nitrogen content in the medium is lowered (Castenholz and Waterbury, 1989).

Recently, it has been shown that the heterocyst differentiation is controlled by a small diffusible peptide (Yoon and Golden, 1998). In heterocyst N_2 fixation is accomplished with the help of nitrogenase enzyme (Fleming and Haselkorn, 1973; Bothe, 1982). Cyanobacteria create an O_2 free environment inside the heterocysts and the nitrogenase that catalyses the reduction, of N_2 to NH_4 is very sensitive to O_2 and poisoned by it. The thick wall of heterocyst is thought to reduce the diffusion of atmospheric gases into the cell to such an extent that all the O_2 is used up in the respiration, although sufficient N_2 enters to saturate the nitrogenase (Fay, 1983). A further adaptation of heterocysts is the loss of O_2 evolving

component of the photosynthetic apparatus, PS II (including the phycobiliproteins). PS I is retained and in the presence of light generates the energy in the form of ATP and reducing power necessary for the reduction of N_2. Additional energy and reducing power are provided by dark respiration, using the O_2 that seeps into the heterocyst. Carbon is imported from adjacent undifferentiated vegetative cells in the form of low molecular weight sugars. Thus vegetative cells and heterocyst need to function in harmony and towards a biochemical symbiosis. Filaments of heterocystous cyanobacteria represent one of the finest examples of division of labour. *i.e.*, vegetative cell supplying the photosynthate to heterocyst and the latter returning the favour in the form of fixed N_2 (Haselkorn, 1978; Stewart, 1980; Apte, 1992).

Heterocysts also plays an important role in reproduction by *in situ* germination (Anand, 1980). In some cyanobacteria, they regulate sporulation (Wolk,1965a,b, 1982; Singh *et al.*,1972; Anand, 1978).

2.4. NITROGEN FIXATION

On dry weight basis, N_2 is the fourth most abundant nutrient element in plants. It is an essential constituent of proteins, nucleic acids, hormones and chlorophyll of cyanobacteria. The bulk of the atmosphere, 78% by volume, consists of molecular N_2, an odourless, colourless gas. Inspite of its abundance, it is scarce in the soil owing to the inability of eukaryotes to fix atmospheric N_2 due to its high energy triple bond (Hopkins, 1995). But some microorganisms including cyanobacteria with the help of some specialized

systems can fix atmospheric N_2. It fixes nitrogen with the help of the nitrogenase enzyme, which is coded by a set of genes called *nif* genes present in the nitrogen fixing sites. Heterocysts are found exclusively in some members of cyanobacteria of the order Nostocales and Stigonematales. Now it is known that some non heterocystous cyanobacteria can also fix atmospheric N_2 in microaerophilic environment.

N_2 fixation is catalyzed by an enzyme complex known as nitrogenase (dinitrogenase). Only prokaryotic cells possess this enzyme and are able to fix N_2 because only they have the gene coding for the enzyme. It is a protein complex made up of two proteins of different size. The smaller protein is a dimer called Fe protein and the larger protein is a tetramer called MoFe protein (Hallenbeck, 1987). The overall reaction for reduction of dinitrogen to ammonia by dinitrogenase is shown in the following equation.

$$8H^+ + 8e^- + N_2 + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\ Pi$$

The source of ATP for cyanobacterial nitrogenase is through photophosphorylation (Cox and Fay, 1969; Bottomley and Stewart,1976; Stewart, 1980; Fay, 1981). However, oxidative phosphorylation can support ATP pool for only short periods of darkness. Further, N_2 fixation in heterocysts is likely to be governed by local ATP pool rather than the pool of adjoining vegetative cells (Burris, 1971).

The most abundant forms of nitrogen present in both terrestrial and aquatic habitats are the inorganic forms of dinitrogen (N_2) such as nitrate

(NO_3^-) and ammonium (NH_4^+) in decreasing order of availability. Thus cyanobacteria can use nitrate or ammonium as the suitable nitrogen source for growth and may have additional ability to fix dinitrogen. In N_2 fixing systems, ammonium will inhibit the action of dinitrogenase. It also interferes with the energy metabolism of the cells, especially ATP production. Thus cyanobacteria avoid any toxicity problem of ammonium by rapidly incorporating ammonium into aminoacids (glutamine). This is accomplished by glutamate synthase cycle with the help of enzyme glutamine synthetase (GS) (Both *et al.*, 1984). BNF, the enzyme nitrogenase catalyzed process by which diazotrophs can reduce N_2 to NH_3, the process is of fundamental importance in supplementing the availability of utilizable N_2 in the biosphere (Howarth *et al.*, 1988; Paerl,1990).

Nitrogen fixed by the cyanobacteria is released either through exudation or through microbial decomposition after the death of cyanobacteria. In paddy fields, the death of cyanobacterial biomass is most frequently associated with soil desiccation at the end of the cultivation cycle and the cyanobacterial growth has frequently resulted in a gradual build up of soil fertility with a residual effect. Nitrogen uptake studies by Wilson *et al.* (1980) shows that 39% of the nitrogen from ^{15}N labelled *Aulosira* sp. spread on soil and 51% from the cyanobacteria incorporated into the soil was recovered in the rice crop indicating the readily availability of cyanobacterial nitrogen to the rice crop. Tirol *et al.* (1982) reported that the availability of

nitrogen from cyanobacteria incorporated into the soil ranged between 23-28% for the first crop and between 27-36% for the second crop seasons.

2.5. CYANOBACTERIA AS BIOFERTILIZER

Nitrogen fixing cyanobacteria play a vital role in the maintenance of soil fertility and sustainability in rice field ecosystems. Cyanobacterial inoculation to rice crop was found to be effective in different agroclimatic conditions and soil types. The paddy field ecosystem provides an environment favourable for the growth of cyanobacteria with respect to their requirement for light, water, temperature, humidity and nutrient availability. This may account for the high abundance of cyanobacteria in low land paddy soils than in upland soils in India (Mitra, 1981), Japan (Okuda and Yamaguchi, 1956a,b) and Ukraine (Prikhod 'Kova, 1971). In acidic soils of Kerala, their abundance varied between 0-76% of the total algae (Aiyer, 1965). Tropical conditions ensure increased incidence of the cyanobacteria in the rice field soils because of high humidity, temperature and shade provided by the crop canopy (Roger and Reynaud, 1979).

The role of nitrogen fixing cyanobacteria in the maintenance of the fertility of rice fields has been well documented (Watanabe and Yamamoto, 1971; Singh, 1978). In India the beneficial effect of cyanobacteria on yield of many rice varieties have been demonstrated in a number of locations (Singh, 1961; Sankaram, 1971; Subrahmanyan, 1972). In submerged soil system, biological nitrogen fixation through cyanobacteria contributes 25 to 30 kg nitrogen per hectre for one cropping season (Venkataraman, 1972). The

beneficial effects of cyanobacterial inoculation on grain yield was well demonstrated by Jaganathan and Kannaiyan (1977), Roger and Kulasooriya (1980), Venkataraman (1981), and Singh and Singh (1985). An average increase of 10-15 per cent in the grain yield could be obtained through cyanobacterial application alone in the case of field experiments and 28 per cent in pot experiments. In experiments, at every low level of fertilizer nitrogen with cyanobacterial supplementation the crop yield was comparable to that in the next higher level of nitrogen, thus compensating the effect of 25 kg nitrogen per hectre (Kaushik, 1980). Under field conditions, supplementation of urea at 60 kg/ha with cyanobacterial inoculation resulted in a grain yield comparable to that obtained with 120 kg nitrogen as urea (Singh *et al.*, 1981).

Many studies have been reported on the use of dried cyanobacteria to inoculate soil as a means of aiding fertility (Roger and Kulasooriya, 1980; Metting, 1988). Cyanobacterial application also reported to increase the height of plant (Singh, 1961), leaf length (Watanabe *et al.*, 1951; Watanabe, 1954) number of tillers (Watanabe, 1962), number of ears (Singh, 1961; Watanabe, 1954), number of spikelets per panicle (Alimagno and Yoshida, 1975). The grain yield, straw yield and nitrogen uptake were increased by 10-12%, 7-10% and 8-37% respectively due to cyanobacterial inoculation without addition of any nitrogen fertilizer.

According to Singh (1981) the cyanobacterial inoculation programme is generally more successful during dry season and cyanobacterial

response with all the precautions are comparable to the application of 20-30 kg N/ha as ammonium sulphate. The cyanobacterial inoculation with composite cultures was found to be more effective than single culture inoculum (Jayaraman, 1990; Kannaiyan, 2001b).

The methodology to prepare inoculum was described by Venkataraman (1981). Usually the inocula are added one week after the rice has been transplanted (Sharma and Gupta, 1983). Ghosh and Saha (1997) reported that inoculation of cyanobacteria during the period when the rice was growing rapidly with a soil based mixture of four heterocystous species resulted in a significant increase in soil nitrogen and total nitrogen uptake by the crop. Although there was only a small increase in grain yield, nitrogen uptake by the grain increased by 30 per cent.

Besides increasing grain yield, cyanobacteria seems to benefit paddy plants by producing growth promoting substances (Chauhan and Gupta, 1984). The presoaked rice seeds with the cyanobacterial culture extracts enhances germination (Gupta and Latha, 1964), promotes the growth of roots and shoots (Gupta and Shukla,1969) and increases the weight and protein content of the grain (Shukla and Gupta, 1967). The probable nature of these substances has been linked to that of gibberellin. The other growth promoting substances produced by the cyanobacteria are vitamins – vitamin B_{12}, folic acid, nicotinic acid, panthothenic acid (Kaushik 2001), IAA, amino acids, etc. (Okuda and Yamaguchi, 1955; Misra and Kaushik, 1989a,b).

Agarwal (1979) claimed that cyanobacteria introduced to the paddy fields can establish themselves permanently if inoculation is done repeatedly for 3-4 cropping seasons. Cyanobacterial inoculation seems to improve soil aggregation due to liberation of polysaccharide (Kaushik and Subhashini, 1985; Roychoudhury *et al.*, 1985), increase organic matter and nitrogen content of inoculated soil (Singh, 1984), in reclamation of Usar soils (Kaushik and Subhashini, 1985), Watanabe (1954) and Kaushik (1985) found that cyanobacterial inoculation enhances the ammonification process thereby increasing the available nitrogen.

Cyanobacterial inoculation of saline and alkaline soils has led to a remarkable decrease in soil pH, electrical conductivity and exchangeable sodium. The sodic soils were converted to calcium soils after application of cyanobacteria for three consecutive cropping seasons. This is due to the secretion of organic acids by cyanobacteria and these acids solubilizes the calcium carbonate in the salt affected soils (Kaushik and Subhashini, 1985; Kaushik, 1987b). It was also reported to increase available phosphorous in the soil due to the excretion of organic acids by the cyanobacteria or the solubilization of unavailable phosphorous (Arora, 1969; Kaushik, 1985). In acidic soils of Kerala, iron and sulphide toxicity is a common phenomenon, algalization greatly reduces the oxidizable organic matter, total sulphide and ferrous ion (Aiyer *et al.*, 1972).

2.6. QUALITY CONTROL OF CYANOBACTERIAL BIOFERTILIZER

Agricultural production depends on the availability and use of quality and quantity of farm inputs. Traditionally Indian agriculture has been depending on natural inputs like farmyard manure, green manure or on microbial activity. The concept of using nitrogen fixing cyanobacteria as a potential biofertilizer in rice fields is not yet fully exploited and some major problems are still limiting the wide utilization of cyanobacteria. The major constraint facing in the biofertilizer technology using cyanobacteria has been the non availability of good quality inoculum at nearby places for the use to the farmers. Another important limitation reported by 45% farmers was the lack of knowledge about quantity and method of use of sticking agent during seed inoculation with biofertilizers (Bhople and Borkar, 2002). The selection of elite cyanobacterial strains has no meaning unless the desired strains are supplied to the farmers. This has necessitated the implementation of better quality control mechanisms to ensure the availability of quality inoculum to the farmers (Kannaiyan, 2001a). The most commonly used cyanobacterial inoculant is soil based. The cyanobacteria were allowed to grow in soil and then it is utilized as inoculum in rice fields. This was adopted by farmers all over India because of its simple technology and economic benefits (Kaushik, 2001). Malliga *et al.* (1996) reported that coir waste as a carrier could increase the shelf life and inoculum potential of the cyanobacterial biofertilizer. Since germination and sporulation process are essential for field application and storage of inoculum, coir waste can be an excellent

carrier material for cyanobacterial biofertilizer and its utilization in paddy fields (Malliga and Subramanian, 2001). Different carrier material such as peat and lignite were used in the preparation of the microbiol inoculants (Somasegaran and Hoben, 1985). A large variety of carriers including thermocol, rice straw, wheat straw and saw dust were also used. The cyanobacteria immobilized in the wheat straw is also found to be very effective that it contains more than 10^5 to 10^6 propagules per gram of carrier. Therefore 1 kg of cyanobacteria per hectre was enough than 10 kg per hectre soil based cyanobacteria as biofertilizer (Kaushik, 2001).

Immobilization enhances the functional and storage longevity of cells (Dainty *et al.*, 1986). Immobilization appears to offer several advantages such as increased biocatalytic capacity due to increased cell densities, stabilization of enzyme activities, the possibility of continuous operation and lower costs of isolation of products. According to Brouers *et al.* (1988) the nitrogenase activity of *Anabaena azollae* was always higher in immobilized cells than in free living cultures. Romo (1997) reported that cells of filamentous cyanobacterium, *Pseudanabaena galeata* were immobilized in sodium alginate beads and stored for 14-18 months and found the structural and functional states of the cells remain unaltered. Kannaiyan (1999) observed that the *Anabaena azollae* immobilized in the polyurethane foam under dried conditions can survive up to one year. According to Bimal and Pandey (2003), the synseeds of four cyanobacterial members (*Gleotrichia echinulata, Rivularia aquatica, Nostoc linckia* and *Anabaena doliolum*) were

packed in polythene bags, sealed and stored at room temperature, 4°C and -20°C for three years. The comparative growth pattern of synseeds observed after three years suggested that all the three cyanobacteria except *Anabaena doliolum* resumed growth after fifteen days of culture. Now a days cyanobacterial immobilization techniques are also being used for the removal and bioaccumulation of metals (Rai *et al.*, 1990; Pant *et al.*, 1992; Pandey *et al.*, 2002).

Materials and Methods

3. MATERIALS AND METHODS

3.1. THE ORGANISMS

The nitrogen fixing cyanobacteria *Scytonema cincinnatum* Thuret ex Born. et Flah. (CU 45294) and *Westiellopsis prolifica* (CU 45286) were isolated from the rice fields of Kerala (Plate 1, 3). The organisms were isolated into unialgal culture by dilution culture followed by cultivating in culture medium $BG_{11}(N^-)$ containing 0.8% (w/v) agar. Pure colonies were transferred to liquid medium and incubated (Plate 2).

3.2. ISOLATION PROCEDURE

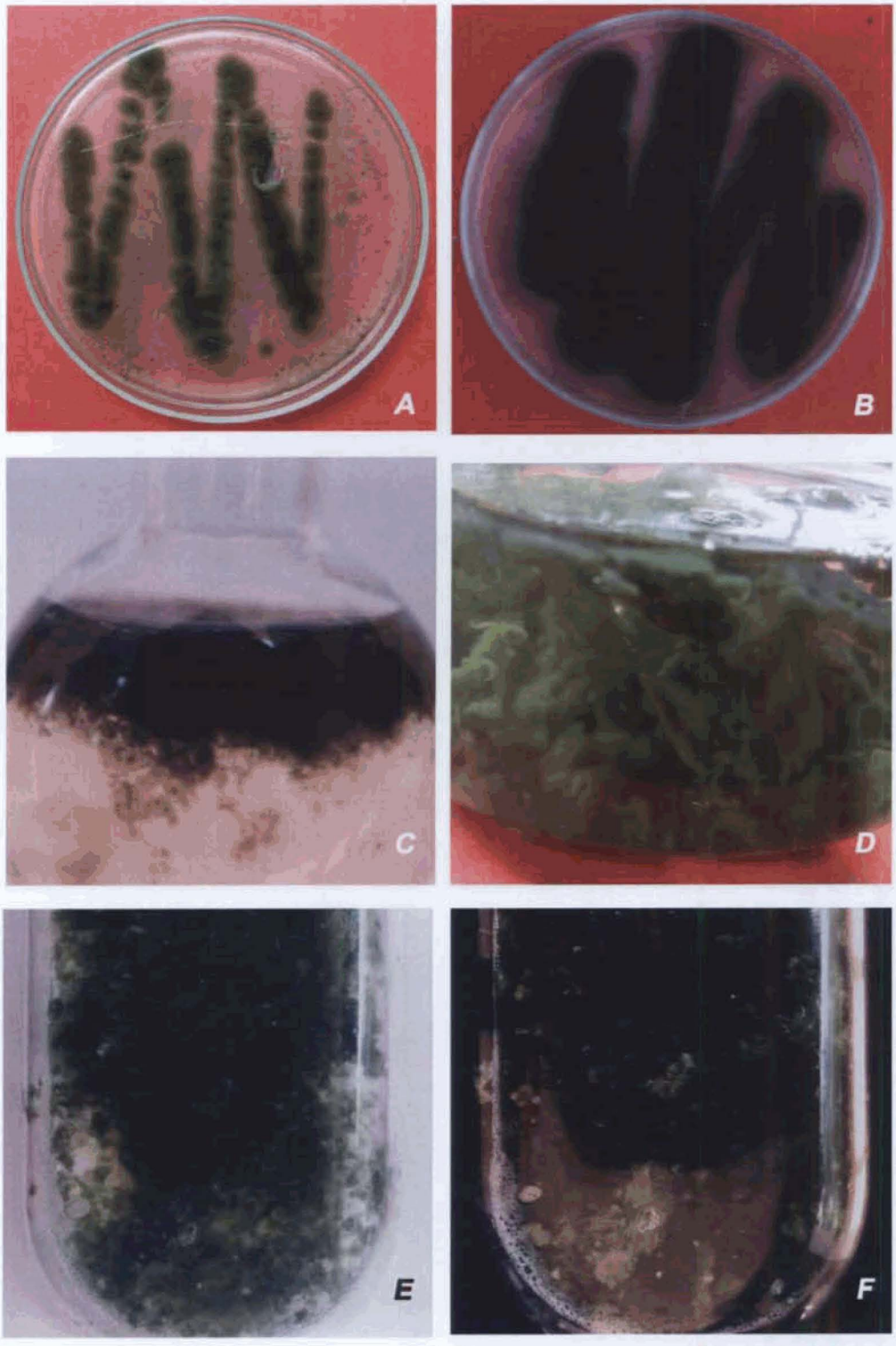
Pure cultures of filamentous forms usually can be obtained simply by repeated liquid transfer of small amount of materials. The collected samples were washed thoroughly in distilled water to remove the soil particles. It was then homogenized using a glass homogenizer. 1ml of the homogenate was made up to 10 ml using distilled water. From this 1ml was taken and made up to 100 ml. This process was repeated 6 – 7 times. Fifth, sixth and seventh dilutions were cultured in liquid medium or on nutrient agar medium in petriplates.

Axenic culture was obtained by exposing the cyanobacteria to low concentrations of antibiotics (Kaushik, 1987a). For checking axenity, the organisms were incubated in following bacteriological test medium.

PLATE 1

A, B, C - Cyanobacterial masses at various locations in the acidic soils of Kerala

PLATE 2



Isolation and culture of W.prolifica and S.cincinnatum in BG - 11 N^- medium

A. Streak on agar plate of S.cincinnatum

B. Streak on agar plate of W.prolifica

C & E - S. cincinnatum in BG - 11 N^- liquid medium

D & F W. prolifica in BG - 11 N^- liquid medium

PLATE 3

A. *Westiellopsis prolifica*
B. *Scytonema cincinnatum*

Dextrose-Peptone Medium

Peptone	:	1%
Dextrose	:	1%
Agar	:	0.8%
D.D.W.	:	100 ml

Peptone was added to water and pH was adjusted to 7.0. Dextrose and agar were added and 10 ml of this was poured into culture tubes. These were sterilized at 10.35 kpa pressure for 15 minutes. After 24 hours the test samples were inoculated into the agar slants under aseptic conditions. The bacterial colonies were not observed even after a week.

The axenic cultures were maintained by repeated subculture of the organisms at a gap of 20 days either in agar medium in petriplates or in liquid BG-11 medium.

3.3. CULTURE METHODS

3.3.1. Culture vessels

Culture tubes (15 x 150 mm, 25 x 100 mm, Borosil) and conical flasks (100 and 250 ml, Borosil and Schott) were used for culturing cyanobacteria. The flasks and tubes were plugged with sterilized non-absorbent cotton plug. The cotton plugs were covered with muslin cloths so that when culture tubes or flasks were tightly covered with cotton plugs, filtered air was allowed to enter in, thus preventing contamination with microbes. The volume of the medium was always maintained about less than half of the culture vessels.

3.3.2. Cleaning Methods

For cleaning purposes the liquid detergents like Teepol/Extran were used. The glassware were immersed in detergents overnight followed by repeated washing in running tap water and finally with distilled water. Glassware were used after drying in hot air oven at 100°C for 5 – 6 hours.

3.3.3. Culture Medium

The Cyanobacteria were grown in nitrogen free BG-11 medium (Rippka *et al.*, 1979). For various experiments BG-11 (N^+ and N^-), Fogg's medium, Allen and Arnon's medium (N^+ and N^-) were also used. The following chemicals (Qualigen/SRL/Merk AR grade) were added at specific concentrations to one litre of distilled water.

BG-11 medium (Rippka *et al.*, 1979)

Chemical	Concentration (g/l)
$NaNO_3$	0.0015
$K_2HPO_4.3H_2O$	0.04
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (di sodium magnesium salt)	0.001
Na_2CO_3	0.02
A_6 micronutrient	1 ml

Micronutrient Solution (A_6)

Chemicals	Concentration (g/l)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_26H_2O$	0.0494

pH of the medium was adjusted to 7.4 before autoclaving

Fogg's Medium (Fogg, 1949)

Chemicals	Concentration (g/l)
KH_2PO_4	0.2
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.1
Fe-EDTA	1 ml
A_5 solution	1 ml

Preparation of Fe-EDTA stock solution (Jacobson, 1951)

Dissolve 26.1g of EDTA in 268 ml of 1N KOH and 24.9 g of ferrous sulphate. Make upto 1 litre. Aerate the solution overnight to produce stable complex marked by change in colour to dark brown.

Micronutrient solution (A_5)

Chemicals	Concentration (g/l)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222

$Na_2MoO_4.2H_2O$	0.0177
$CuSO_4.5H_2O$	0.079

pH of the medium was adjusted to 7.5 before autoclaving.

Allen & Arnon's Medium (Allen and Arnon, 1955)

Chemicals	**Concentration (g/l)**
KNO_3	2.020
$K_2HPO_4.3H_2O$	0.456
$MgSO_4.7H_2O$	0.246
NaCl	0.232
$CaCl_2.2H_2O$	0.074
A5 micronutrient	1 ml
Fe-EDTA	1 ml

For preparing (N^-) mediums $NaNO_3$ and KNO_3 are omitted. The stock solutions of the chemicals were prepared with double distilled water and autoclaved at 1.06 kg/cm^2 pressure for 20 minutes and stored in refrigerator for subsequent use. While preparing the medium, 1ml of the each stock solution was taken and made up to 1 litre by adding distilled water.

For preparing solid media, agar is added to media at concentration of 0.8% (W/V). The media were taken in a test tube or petriplate.

3.3.4. Sterilization

The culture medium and glassware used for experiments were sterilized for 20 minutes in an autoclave under a pressure of 1.06 kg/cm^2.

3.3.5. Culture conditions

The cultures were maintained in culture racks at a temperature of 25 ± 1°C under continuous illumination of 1500-2000 lux for 12 hours light and 12 hours darkness. Because of the high concentration of water in agar, condensation of water may form in petriplates during incubation and moisture is likely to drip from the cover into the surface of the agar and spread out, resulting in a confluent mass of growth and ruining individual colony formation. To avoid this, the petriplates were incubated bottom side up. The slant cultures were kept in slanting position, with the streak towards the light source. The cultures in the liquid medium were hand shaken twice daily to prevent sticking of the cells to the walls of the glass vessels. All petriplates, tubes, and flasks were labelled with name, date and identification of contents.

3.3.6. Inoculation

Inoculation was carried out in a laminar airflow cabinet provided with a UV lamp. The work area of the laminar air flow was wiped with 90% ethanol and were exposed to UV radiation for 30 minutes before use, to avoid contamination from microbes. The requirements needed for inoculation like forceps, sterilized needle, glassware, etc. were also exposed to UV radiations. For experiments the organism were homogenized using a glass homogenizer (20 ml capacity). Equal amount of inoculum suspension was pipetted into each experiment vessel containing medium.

3.3.7. pH adjustments

The pH of the culture medium was adjusted to 7.4 – 7.5 using 0.1N HCl/NaOH using a Systronics digital pH meter (Model 335).

3.4. ANALYTICAL METHODS

3.4.1. Estimation of chlorophyll - a

Chlorophyll-a was assayed according to Parsons and Strickland (1965) method. The culture suspension of cyanobacterium was homogenized and centrifuged at 5000 rpm for 10 minutes (Model Remi R8C). The supernatant was discarded and the pellet was washed twice in distilled water. The resulting pellet was suspended in a known volume of 80% (v/v) acetone (SRL, AR) and incubated in dark refrigerator for 20 – 24 hours. After the extraction period, the samples were taken out from the refrigerator and allowed them to warm to room temperature. If the solvent had evaporated, it was made up to known volume by adding 80% acetone. The acetone extract was separated by centrifugation at 5000 rpm for 10 minutes. Absorbance of the acetone extract was measured at a wavelength of 665 nm, 645 nm, and 630 nm using a Spectrophotometer (Model Thermo Spectronic Genesys 20). The equation used for the estimation of the concentration of chlorophyll-a (Ca) in mg/ml at absorbance (D) is

$$Ca = 11.6\ D_{665} - 1.31\ D_{645} - 0.14\ D_{630}$$

3.4.2. Estimation of carotenoids

Carotenoids were estimated according to Siegelman and Kycia (1979). The suspension of cyanobacterium was centrifuged at 5000 rpm for 10 minutes. The supernatent was discarded and the pellet was suspended in a known volume of 85% (v/v) acetone (SRL/AR) and kept in dark refrigerator for 20 – 24 hours. After the extraction period, the samples were taken out from the refrigerator. If the solvent had evaporated, it was made up to known volume by adding 85% acetone. The absorbancy (D) were taken at 450 nm against acetone blank. The total amount of carotenoids in mg/ml was estimated by using the formula.

$$\text{Carotenoids (C)} = \frac{D \times V \times F \times 10}{2500}$$

Where V is the volume of the sample, F is the dilution factor and the value 2500 is the average extinction coefficient of the pigment.

3.4.3. Estimation of Phycobiliproteins

Phycobiliprotein was assayed according to Bennet and Bogorad (1971) method. The culture suspension was harvested by centrifugation at 5000 rpm for 10 minutes. The pellet was washed twice in distilled water and suspended in 3 ml of 0.05 M phosphate buffer containing equal volumes of 0.1 M solutions of KH_2PO_4 and K_2HPO_4 maintained at pH 6.7. Freeze the pellet at 5°C. After removing the culture suspension from refrigerator, the cells were thawed with a mortar and pistle using sand as an abrasive. It was

repeated several times to ensure complete extraction. Centrifuged the suspension. The absorbance of the supernatent were taken at wave lengths of 562 nm, 615 nm and 652 nm and calculated concentrations of c-phycoerythrin (PE), c-phycocyanin (PC), and allophycocyanin (APC) using the equations derived from extinction coefficients of purified phycobiliproteins.

c – phycocyanin (PC) = $A_{615} - 0.474\ (A_{652})/5.34$

Allophycocyanin (APC) = $A_{652} - 0.208\ (A_{615})/5.09$

c – phycoerythrin (PE) = $A_{562} - 2.41$ (PC) – 0.849 (APC)/9.62

3.4.4. Estimation of proteins

The proteins of the experimental cultures was estimated according to Lowry *et al.* (1951) and Price (1965). The culture suspension was homogenized and centrifuged at 5000 rpm for 10 minutes. The pellet was washed twice in distilled water. To the pellet added 5 ml of 10% TCA and left for one minute, after this, again centrifuged and removed the filtrate. To the pellet added 5 ml of alkaline reagent (2% Na_2CO_3 in 0.1N NaOH + 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate) and incubated for 10 minutes. To this added 0.5 ml of 1N Folin-Ciocalteau reagent, mixed thoroughly and incubated at room temperature in dark for 30 minutes. A blue colour so developed was measured at 750 nm in a spectrophotometer against the reagent blank.

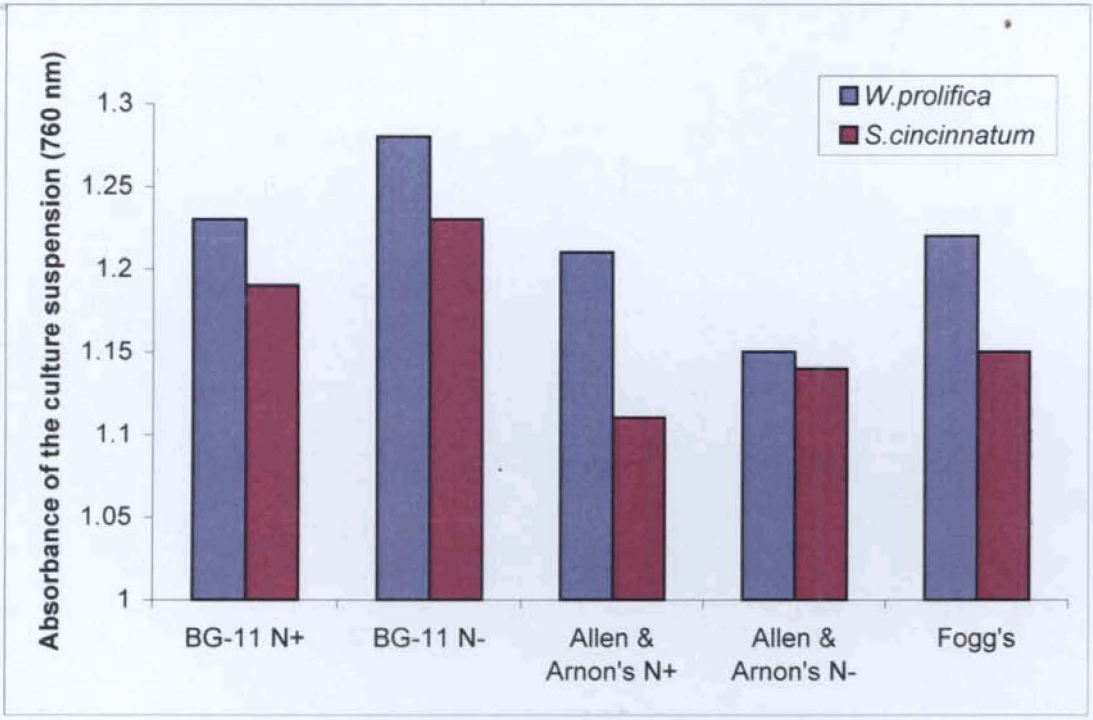


Fig. 1 Effect of different media on the growth (absorbance of the culture suspension at 760nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation

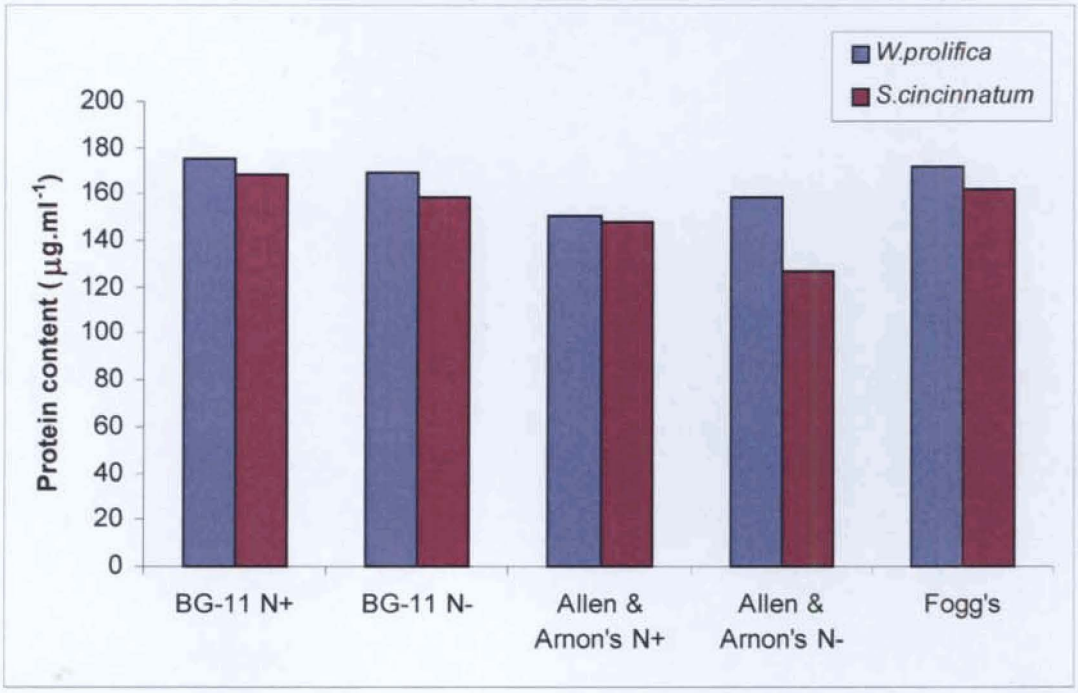


Fig. 2 Effect of different media on protein content of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation

The amount of protein of the sample was calculated from the standard curve prepared by using Bovine Serum Albumin taken with in a range of 1-100 μg/ml.

3.4.5. Estimation of ammonia

The release of ammonia by cyanobacterial culture was estimated by phenol-hypochlorite method with a modification in pH of the reagents (Solorzano, 1969).

Reagents

(i) Alkaline solution (pH 10)

Trisodium citrate	-	20g
NaOH	-	5 g
D.D.W.	-	100 ml

Freshly prepared at the start of the experiment.

(ii) Sodium hypochlorite stock

Sodium hypochlorite with 4% available chlorine.

(iii) Nitroprusside reagent (pH 10)

Sodium nitroprussiode	-	500 mg
D.D.W	-	100 ml

(iv) Phenol reagent

Distilled phenol	-	10 g

95% ethanol - 100 ml

Prepared and stored in amber coloured bottle.

(v) Oxidizing reagent

Alkaline stock and sodium hypochlorite stock mixed in the ration 4:1 freshly before use.

Procedure

The cyanobacteria grown in BG-11 medium is filtered and the filtrate was used for the estimation of ammonia excretion. 5ml of the culture filtrate was mixed with 200 µl of nitroprusside reagent and mixed well. Then 500 µl of the oxidizing reagent was added. The tubes were incubated in darkness for 1 hour with intermittent shaking. The blue colour developed was measured at 620 nm in a spectrophotometer against the blank. The standard graph was prepared with 100-1000 n moles ml^{-1} of NH_4Cl and the results were expressed in n moles of ammonia excreted per ml of the culture filtrate.

3.4.6. Growth measurements

(a) By Optical density

The growth was estimated by measuring the absorbance of the homogenized culture suspension at a wavelength of 760 nm (Adhikary, 1983) in a spectrophotometer against a reagent blank of sterile BG-11 medium.

(b) Growth rate

The growth rate (K) was determined by means of generation time (G). It is the time interval required for the cell to divide or for the population to double. The generation time varies from organism to organism and also depends on the amount and kind of nutrients available in the medium and the physical conditions which prevail in the growing medium. It was determined by the formula given by Guillard (1973).

$$K = \ln (N_1/N_0) (1.443/t)$$

Where N_1 and N_0 are the cell concentrations at the end and beginning of a period time t days. From K, the generation time can also calculated by

$$Td = 1/K \text{ days per division} = 24/K \text{ hours per division.}$$

3.4.7. Heterocyst abundance (Kaushik, 1987a)

The cyanobacteria were placed on a glass slide and examined under microscope. Number of heterocysts per 100 vegetative cells were counted. The heterocyst abundance was expressed as percentage over total population of cells.

$$\% \text{ of heterocyst} = \frac{\text{Total No. of Heterocysts}}{\text{Total No. of Vegetative Cells}} x100$$

3.4.8. Morphology

The morphology was studied by observing the cyanobacteria through a light microscope and the measurements were taken using an ocular micrometer.

All the experiments were conducted in test tubes stoppered with non absorbant cotton wool plug containing 10 ml of nitrogen free BG-11 medium with or without of various concentrations of chemicals and the organisms. Equal amount of inoculum was added to all the tubes and the initial absorbance of the culture suspension at 760 nm soon after inoculation was made into 0.2 in most of the experiments. The organisms were incubated up to 42 days and the readings were taken at an interval of 7 days.

3.4.9. Statistical Analysis

Triplicates were set up for each set of experiments. Mean value of triplicate determinations $\pm$ S.D was calculated and presented in the text.

3.5. EXPERIMENTAL PROCEDURE

For growth experiments, aliquots of equal amount of exponentially growing organisms was inoculated into cotton stoppered hard glass test tubes containing 20 ml of BG-11 (N^+ and N^-) medium, Fogg's medium and Allen and Arnon's (N^+ and N^- medium). It was incubated up to 30 days and then harvested.

For pH experiments, equal amount of exponentially growing cyanobacteria was inoculated into 10 ml BG-11 N^- and incubated up to 42 days. The different pH levels (3.0, 4.0, 5.0, 6.0, 7.0, 7.4, 8.0, 9.0) of the culture medium was obtained by addition of 0.1 N HCl and 0.1 N NaOH to the medium aseptically. Absorbance of the culture suspension at 760 nm of each organism soon after inoculation into the experimental tube was 0.2. The pH of the medium was regularly maintained till the end of the experiment.

In order to examine the response of cyanobacteria to various concentrations of NaCl, stock solution of the salt was prepared in distilled H_2O, sterilized separately and then added aseptically to the culture medium to obtain desired concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 M). Equal amount of each organisms were inoculated (absorbance of the culture suspension at 760 nm soon after inoculation was 0.3) and incubated up to 42 days.

For studying autotrophic, mixotrophic and chemoheterotrophic growth capability, three sugars (sucrose, glucose, fructose) were supplemented to BG-11 N^- medium at a final concentrations of 15 mM in the culture. For this experiment the absorbance of the culture suspension soon after inoculation at 760 nm was 0.2. Cultures were incubated under continuous light or in the dark for 15 days and then their growth was estimated.

For studying the effect of various agrochemicals, organic pesticides and PGRs like carbofuran, neem and 2,4-D, stock solutions were prepared

in distilled water, sterilized separately and then added aseptically to the culture medium to obtain the desired concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 ppm). Equal amount of organisms were inoculated (absorbance of the culture suspension at 760 nm soon after inoculation was 0.2) and incubated up to 42 days.

For studying the effect of various chemical fertilizers such as nitrogen (urea and ammonium sulphate), phosphorus (super phosphate) and potassium (potash and potassium sulphate), the stock solutions were prepared separately in distilled water and added aseptically to the culture medium to obtain the desired concentrations of 1, 5, 10 and 20 ppm respectively. Equal amount of exponentially growing cultures were inoculated into the medium and harvested after 28 days.

Immobilization was carried out by adding 3% (W/V) sodium alginate (BDH) to $CaCl_2$ free mediums (BG-11 N^+ and N^-; Fogg's, Allen and Arnon's N^+ and N^-). For immobilization, $CaCl_2$ at 50 mM prepared in double distilled water and used. Medium containing sodium alginate and $CaCl_2$ solutions were sterilized in an autoclave and the encapsulation was carried out in aseptic conditions.

Highly concentrated cyanobacterial suspension obtained after centrifuging 20 days old cultures were taken for the present study. Cyanobacteria were washed 2-3 times in double distilled water and filtered. It was then transferred to a homogenizer and homogenized well in respective medium. Then it was transferred to sodium alginate containing

medium. Using a sterile pipette of 1.0 cm diameter at the tip, the alginate solution containing the cyanobacteria were drawn and dropped into $CaCl_2$ solution and kept there for 15-30 minutes. $CaCl_2$ solution was then decanted off and the beads were washed repeatedly (3 times 5 minute each) using double distilled water. It was then washed with respective medium and finally transferred to $CaCl_2$ free solid and liquid mediums.

Results and Discussion

4. RESULTS AND DISCUSSION

4.1. EFFECT OF DIFFERENT MEDIA ON THE GROWTH OF *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM*

4.1.1. Results

In order to determine the suitable growth medium for their (Cyanobacterial strains) diazotrophic growth for further experiments, equal amount of inoculum of each cyanobacterial strain was inoculated in six standard culture media *viz.*, BG-11 N^- medium, BG-11 N^+ medium, Allen and Arnon's N^- medium, Allen and Arnon's N^+ medium and Fogg's medium. The cultures were grown under photoautotrophic growth conditions for 30 days. Their growth was determined in terms of optical density and along with this protein content, chlorophyll-a content, carotenoid content and excretion of ammonia was also calculated. The results presented in Table & Fig. 1-5 reveal that N_2-fixing capacity of both the cyanobacteria was relatively better in BG-11 N^- medium than the other four media. Both strains withstood in all the five media but *Westiellopsis prolifica* and *Scytonema cincinnatum* could not survive in Allen and Arnon's N^- medium and Allen and Arnon's N^+ medium respectively, and after one month of growth and they lost its green colour.

Based on the above experiments, it was decided to grow all cyanobacterial strains in BG-11 N^- medium for conducting further experiments.

Table 1. Growth response (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* to different media on 30th day of incubation at 25 ± 1°C with 12 h light/dark cycle

Media	*Westiellopsis prolifica*		*Scytonema cincinnatum*	
	Absorbance at 760 nm	Generation time	Absorbance at 760 nm	Generation time
BG-11 N^-	1.28 ± 0.06	14	1.23± 0.03	15
BG-11N^+	1.23 ± 0.05	15	1.19 ± 0.07	16
Allen & Arnon's N^-	1.15 ± 0.07	17	1.14 ± 0.05	17
Allen & Arnon's N^+	1.21 ± 0.05	15	1.11 ± 0.06	21
Fogg's	1.22 ± 0.04	15	1.15 ± 0.03	17

Values represent mean of three replicates ± S.D.

Table 2. Protein content (μg.ml^{-1}) of *Westiellopsis prolifica* and *Scytonema cincinnatum* in different media on 30th day of incubation at 25 ± 1°C with 12h light/dark cycle

Media	*Westiellopsis prolifica*	*Scytonema cincinnatum*
BG-11N^-	175.23 ± 0.09	168.14 ± 0.06
BG-11 N^+	169.44 ± 0.07	159.27 ± 0.05
Allen & Arnon's N^-	151.42 ± 0.09	148.26 ± 0.08
Allen & Arnon's N^+	159.19 ± 0.11	127.31 ± 0.07
Fogg's	172.33 ± 0.08	162.13 ± 0.09

Values represent mean of three replicates ± S.D.

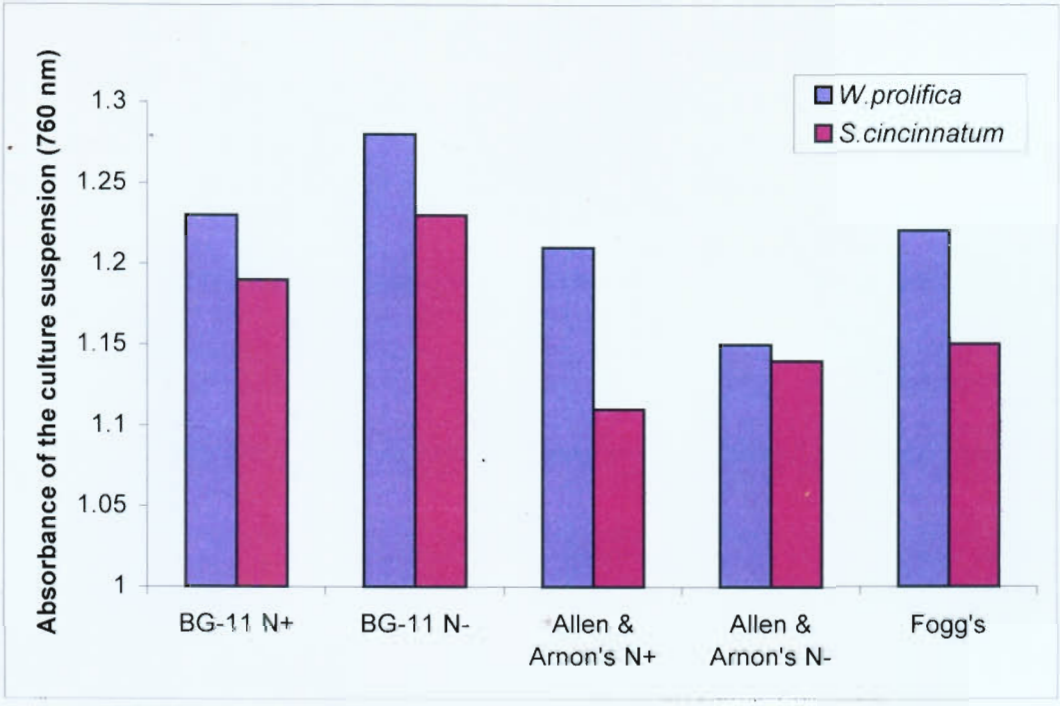


Fig. 1 Effect of different media on the growth (absorbance of the culture suspension at 760nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation

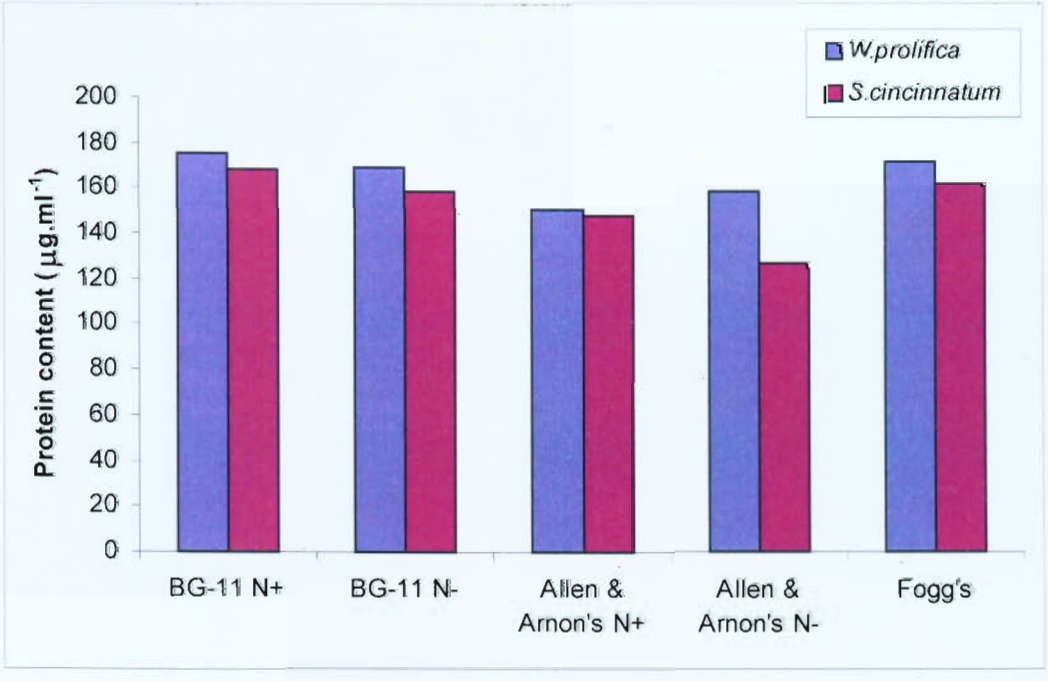


Fig. 2 Effect of different media on protein content of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation

Table 3. Chlorophyll-a content ($\mu g.ml^{-1}$) of *Westiellopsis prolifica* and *Scytonema cincinnatum* in different media on 30th day of incubation at 25 ± 1°C with 12 h light/dark cycle.

Media	*Westiellopsis prolifica*	*Scytonema cincinnatum*
BG-11N$^-$	13.42 ± 0.03	10.5 ± 0.01
BG-11 N$^+$	11.14 ± 0.02	8.5 ± 0.03
Allen & Arnon's N$^-$	7.02 ± 0.02	1.41 ± 0.03
Allen & Arnon's N$^+$	10.54 ± 0.03	0.82 ± 0.02
Fogg's	6.18 ± 0.5	8.10 ± 0.03

Values represent mean of three replicates ± S.D.

Table 4. Carotene content ($\mu g.ml^{-1}$) of *Westiellopsis prolifica* and *Scytonema cincinnatum* in different media on 30th day of incubation at 25 ± 1°C with 12 h light/dark cycle

Media	*Westiellopsis prolifica*	*Scytonema cincinnatum*
BG-11N$^-$	3.16 ± 0.11	1.96 ± 0.10
BG-11 N$^+$	3.01 ± 0.10	1.81 ± 0.10
Allen & Arnon's N$^-$	2.69 ± 0.09	1.52 ± 0.08
Allen & Arnon's N$^+$	2.95 ± 0.10	0.19 ± 0.09
Fogg's	2.92 ± 0.08	2.73 ± 0.08

Values represent mean of three replicates ± S.D.

Table 5. Ammonia excretion (n moles ml^{-1}) by *Westiellopsis prolifica* and *Scytonema cincinnatum* in different media on 30th day of incubation at 25± 1°C with 12 h light/dark cycle

Media	*Westiellopsis prolifica*	*Scytonema cincinnatum*
BG-11N$^-$	56 ± 0.15	49 ± 0.13
BG-11 N$^+$	41 ± 0.16	33 ± 0.12
Allen & Arnon's N$^-$	38 ± 0.14	30 ± 0.11
Allen & Arnon's N$^+$	32 ± 0.15	20 ± 0.14
Fogg's	40 ± 0.13	36 ± 0.11

Values represent mean of three replicates ± S.D.

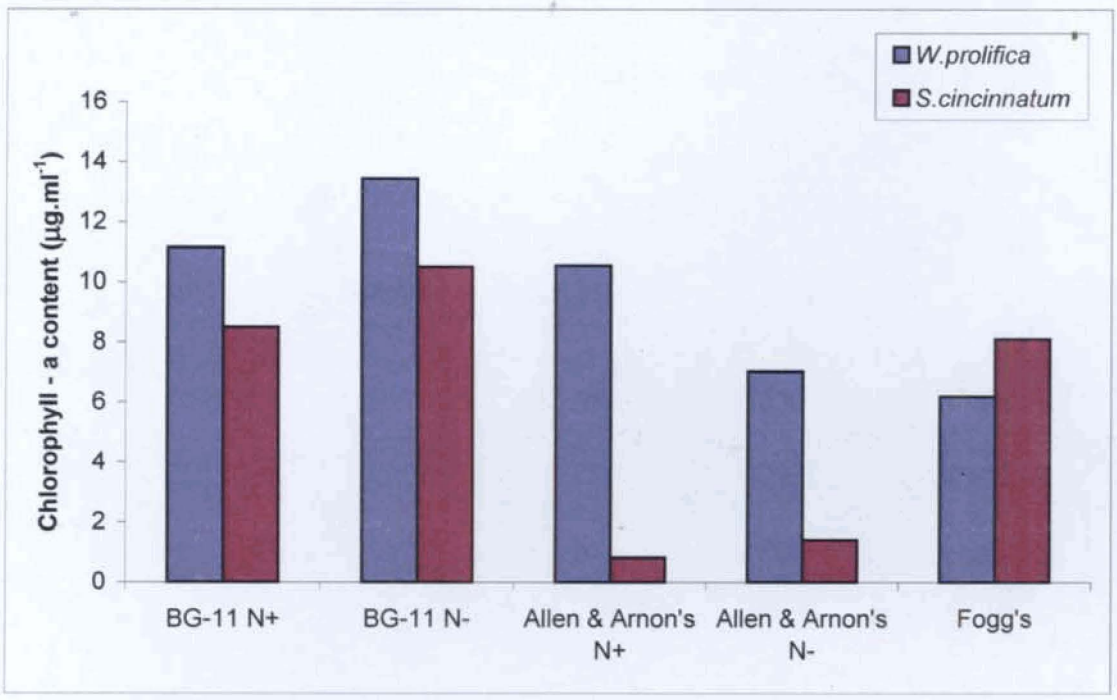


Fig. 3 Effect of different media on chlorophyll-a content of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation.

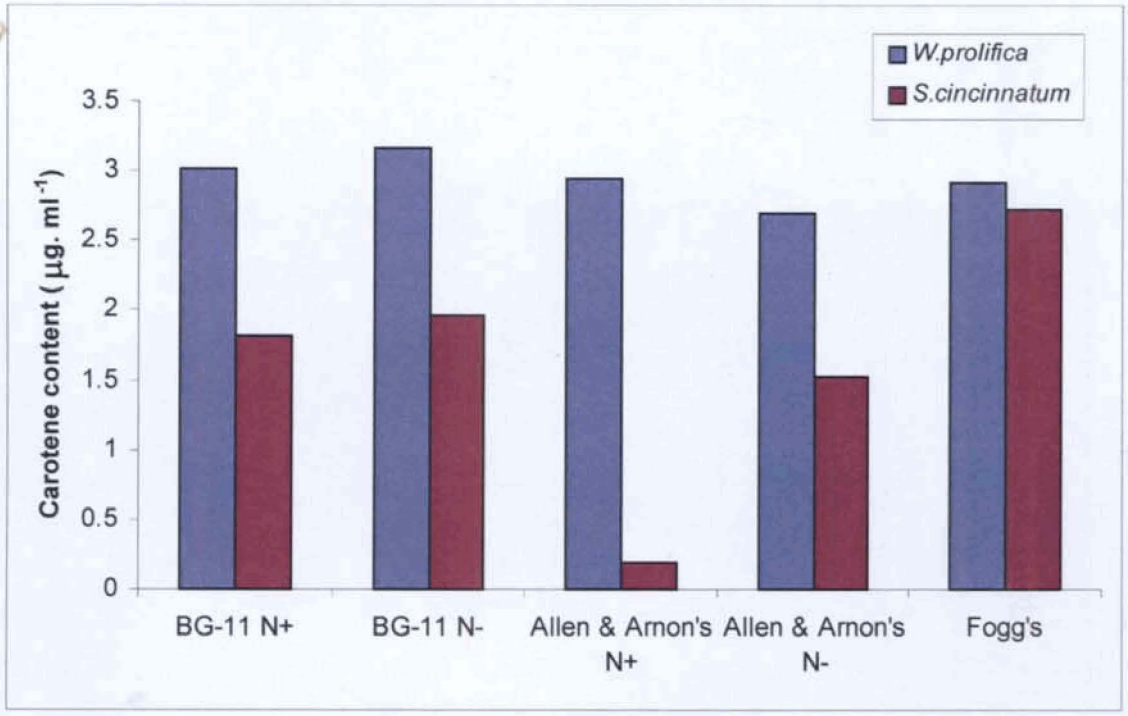


Fig. 4 Effect of different media on carotene content of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation.

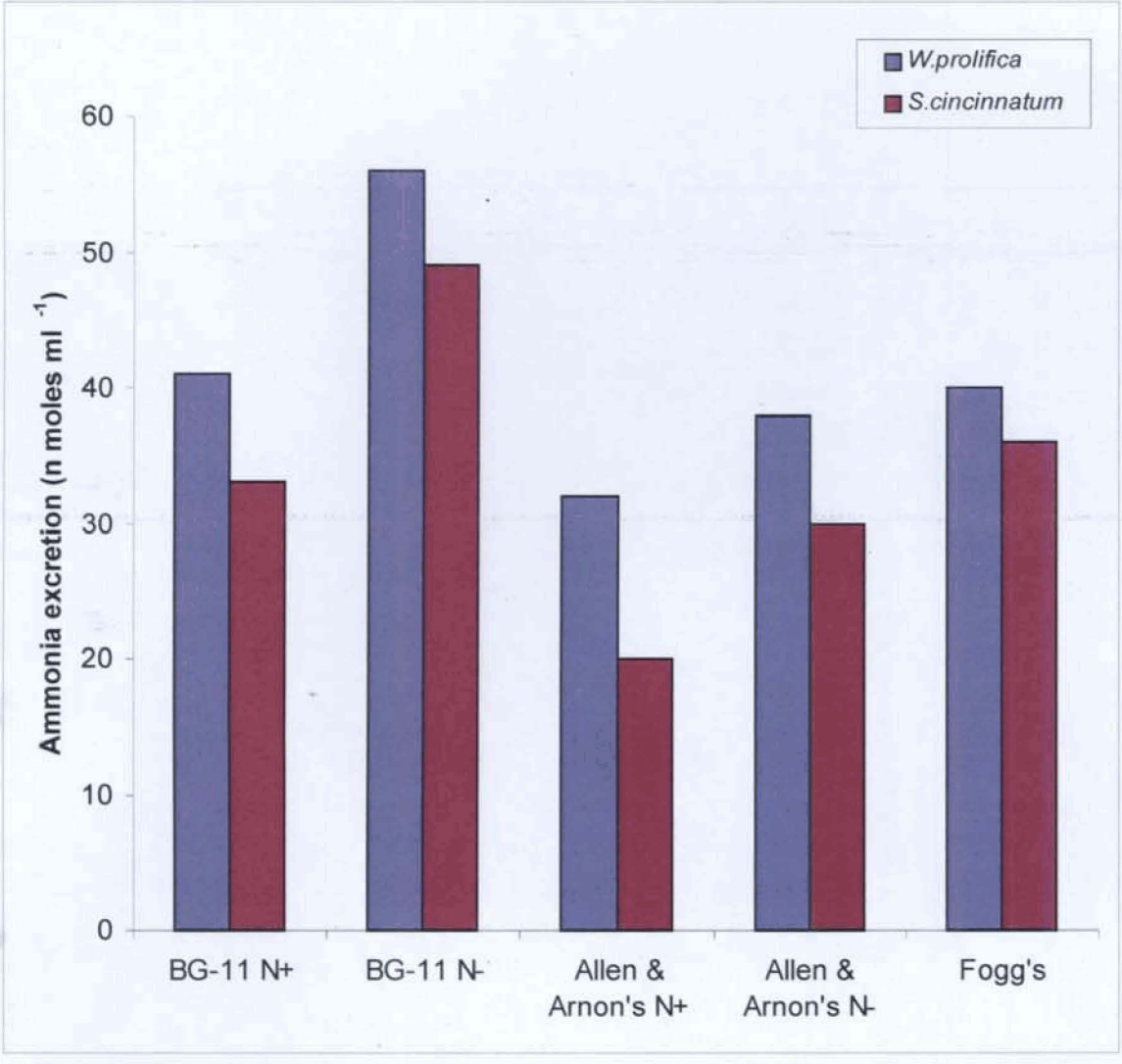


Fig. 5 Effect of different media on ammonia excretion of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 30th day of incubation.

4.2. GROWTH CHARACTERISTICS AND AMMONIA EXCRETION OF CYANOBACTERIA AT VARIOUS pH LEVELS OF THE CULTURE

4.2.1. Results

Growth response (absorbance of the culture suspension at 760 nm) as well as protein, chlorophyll-a content, phycobiliprotein content and ammonia excretion of *Westiellopsis prolifica* CU 45286 and *Scytonema cincinnatum* CU 45294 at different pH levels of the culture was studied. The organisms were grown at pH 3.0, 4.0, 5.0, 6.0, 7.0, 7.4 (control), 8.0 and 9.0 in the culture for a period of 42 days and they are harvested at an interval of 7 days. Of the two species, both of them showed increased growth, chlorophyll-a content, protein content, phycobiliprotein content and ammonia excretion in the alkaline pH of the medium up to pH 9.0. *Westiellopsis prolifica* showed good growth in the acidic range of pH (pH 3-6) but the growth increased significantly in alkaline range with maximum at pH 8. At pH 9, growth slightly decreased (Table & Fig. 6). Similarly *Scytonema cincinnatum* also showed fairly good growth in all the pH ranges from pH 3.0 to 9.0 with maximum growth in the alkaline range at pH 7.4. The growth slightly decreased at the pH 8.0 and 9.0 when compared with the control (Table & Fig. 7). The protein content of *Westiellopsis prolifica* increased with increase in pH and the maximum was noticed at pH 8.0 (Table & Fig. 8). In *Scytonema cincinnatum* the protein content increased from acidic to alkaline range with maximum protein content at pH 7.4 and the protein content at the

acidic range was comparatively lesser than the alkaline range (Table & Fig. 9). The chlorophyll-a of *Westiellopsis prolifica* was low at pH 3.0, 4.0, 5.0 and 6.0 and increased with increase in pH and was maximum at pH 8.0. At pH 9.0 the chlorophyll-a decreased slightly (Table & Fig. 10). At pH 3.0 to 6.0 *Scytonema cincinnatum* showed very little increase in the chlorophyll-a content from the 7^{th} day to the end of the experiment. But the chlorophyll-a increased in the alkaline range and the increase was tremendous after 14^{th} day of incubation. Chlorophyll-a slightly decreased at pH 9.0 (Table & Fig. 11). The carotene content of *Westiellopsis prolifica* increased in all the ranges of pH with maximum at pH 8.0 (Table & Fig. 12). In *Scytonema cincinnatum* the carotene content levelled off at acidic ranges of pH and it increased suddenly at alkaline pH range and was maximum at pH 7.4 (Table & Fig. 13). The phycobilin content of *Westiellopsis prolifica* was maximum at pH 8.0 (Table & Fig. 14-16) and at pH 7.4 for *Scytonema cincinnatum* (Table & Fig. 17-19). The cyanobacteria showed continuous excretion of ammonia from acidic to alkaline range and the excretion was maximum at pH 8.0 for *Westiellopsis prolifica* and at pH 7.4 for *Scytonema cincinnatum* (Table & Fig. 20). The heterocyst frequency of *Westiellopsis prolifica* was maximum at pH 8 showing 13.9% and lowest at pH 3 showing 4.6% while in *Scytonema cincinnatum* maximum heterocyst frequency was at pH 7.4 showing 8.9% and was lower at pH 3.0 showing 4.1% (Table & Fig. 21, 22).

Table 6. Effect of pH on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* upto 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	0.21 ± 0.14	0.22 ± 0.06	0.23 ± 1.71	0.25 ± 0.79	0.30 ± 2.31	0.32 ± 0.19
pH 4	0.21 ± 0.23	0.22 ± 0.14	0.25 ± 2.32	0.26 ± 0.83	0.34 ± 2.16	0.40 ± 0.26
pH 5	0.21 ± 0.12	0.23 ± 0.27	0.26 ± 0.93	0.28 ± 2.37	0.41 + 2.32	0.45 ± 0.91
pH 6	0.21 ± 0.94	0.24 ± 1.21	0.28 ± 0.74	0.31 ± 3.42	0.48 ± 1.36	0.55 ± 2.32
pH 7	0.22 ± 0.64	0.25 ± 2.71	0.27 ± 0.92	0.32 ± 1.76	0.52 ± 1.92	0.59 ± 1.79
pH 7.4 (Control)	0.26 ± 0.17	0.30 ± 2.61	0.33 ± 0.42	0.43 ± 1.11	0.56 ± 1.81	0.72 ± 1.83
pH 8	0.25 ± 0.11	0.27 ± 0.92	0.32 ± 0.19	0.48 ± 1.71	0.59 ± 1.33	0.84 ± 1.96
pH 9	0.20 ± 0.14	0.22 ± 0.86	0.25 ± 1.36	0.38 ± 1.35	0.53 ± 2.15	0.78 ± 1.18

Values represent mean of three replicates ± S.D.

Table 7. Effect of pH on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	0.20 ± 0.11	0.21 ± 0.12	0.22 ± 0.11	0.23 ± 0.08	0.25 ± 0.09	0.27 ± 0.07
pH 4	0.21 ± 0.09	0.22 ± 0.09	0.23 ± 0.08	0.25 ± 0.06	0.27 ± 0.08	0.29 ± 0.06
pH 5	0.21 ± 0.06	0.23 ± 0.09	0.24 ± 0.08	0.25 ± 0.07	0.28 + 0.09	0.30 ± 0.07
pH 6	0.22 ± 0.08	0.23 ± 0.08	0.26 ± 0.06	0.27 ± 0.05	0.31 ± 0.06	0.33 ± 0.05
pH 7	0.23 ± 0.12	0.25 ± 0.07	0.27 ± 0.09	0.29 ± 0.06	0.33 ± 0.09	0.35 ± 0.06
pH 7.4 (Control)	0.23 ± 0.08	0.26 ± 0.09	0.29 ± 0.07	0.31 ± 0.09	0.36 ± 0.08	0.38 ± 0.05
pH 8	0.22 ± 0.07	0.24 ± 0.11	0.25 ± 0.05	0.27 ± 0.08	0.30 ± 0.09	0.32 ± 0.09
pH 9	0.21 ± 0.09	0.23 ± 0.13	0.24 ± 0.06	0.25 ± 0.06	0.27 ± 0.06	0.28 ± 0.06

Values represent mean of three replicates ± S.D.

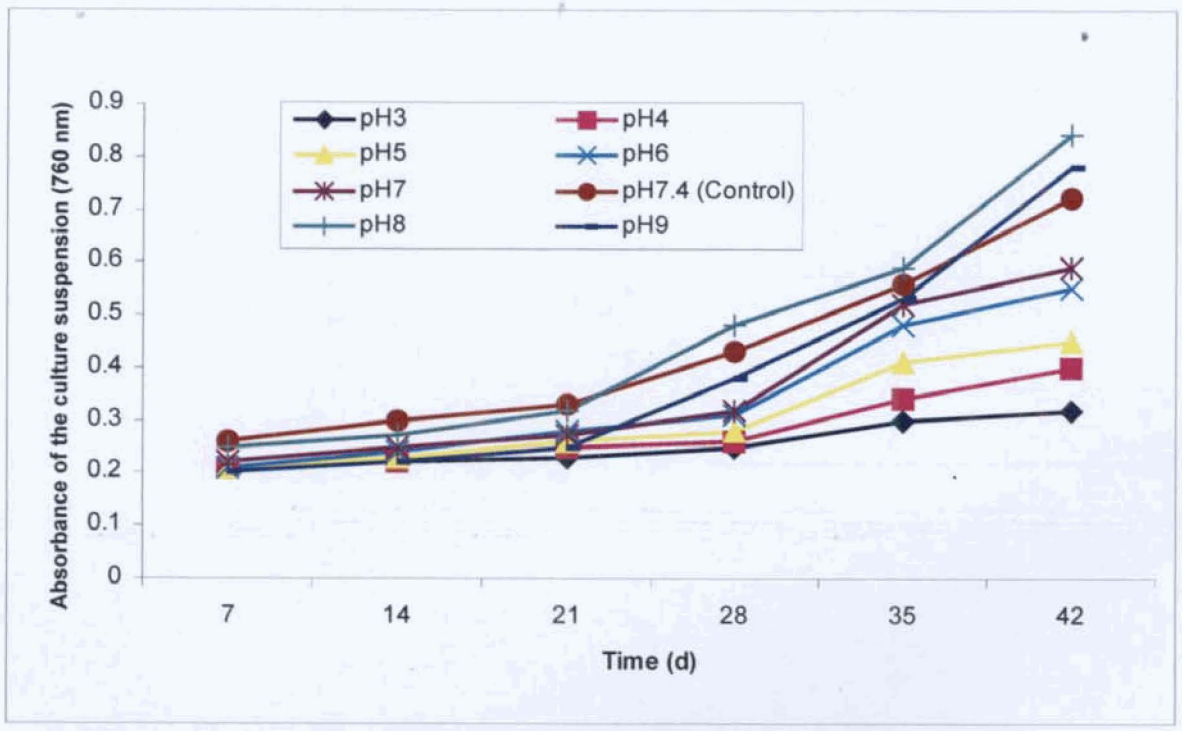


Fig. 6 Effect of pH on the growth (absorbance of the culture suspension at 760nm) of *Westiellopsis prolifica* up to 42 days of incubation.

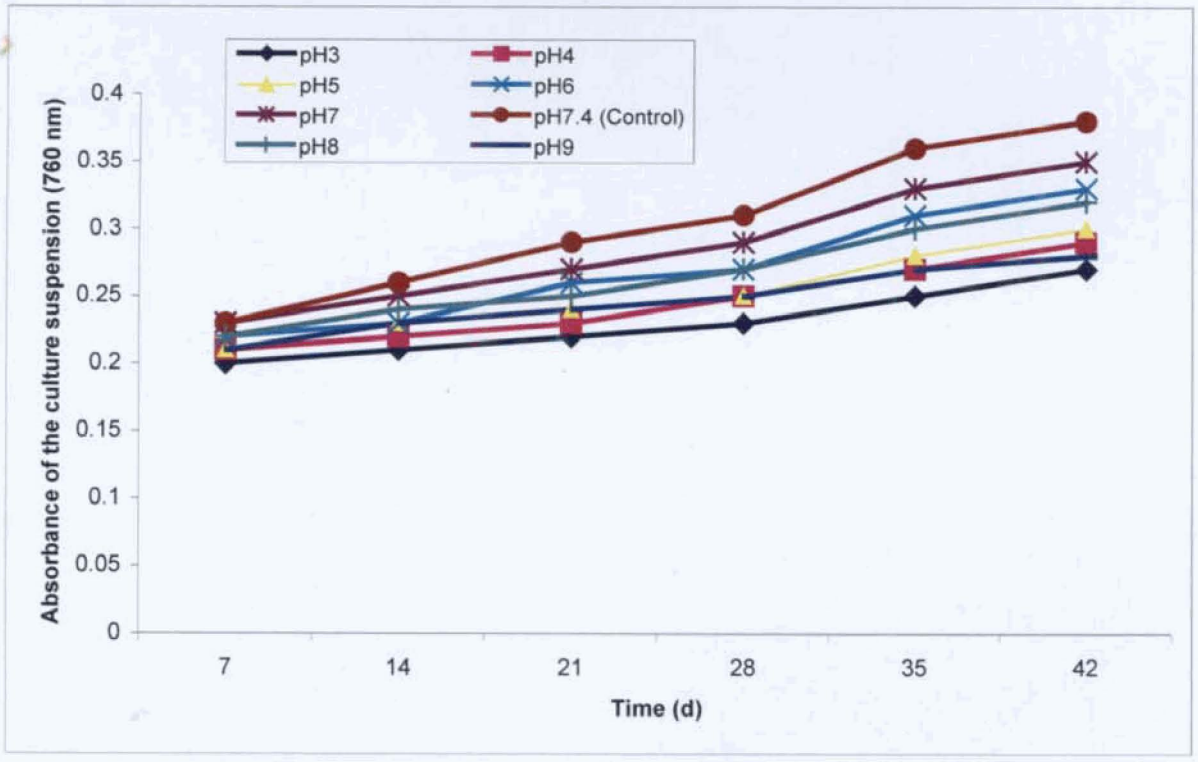


Fig. 7 Effect of pH on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 42 days of incubation.

Table 8. Effect of pH on the protein content (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	26.08 ± 1.61	30.14 ± 1.38	30.89 ± 1.12	31.26 ± 1.82	33.21 ± 3.41	35.67 ± 0.91
pH 4	26.25 ± 1.35	30.55 ± 2.67	31.94 ± 0.09	35.58 ± 1.36	35.26 ± 0.09	38.88 ± 0.43
pH 5	26.96 ± 1.82	31.33 ± 2.12	33.22 ± 1.71	36.16 ± 1.72	39.86 ± 1.72	42.72 ± 0.07
pH 6	27.32 ± 2.37	33.72 ± 2.97	35.16 ± 1.83	49.51 ± 1.79	52.63± 2.71	54.51 ± 1.16
pH 7	27.50 ± 1.93	34.66± 2.18	36.72 ± 2.13	51.11 ± 1.63	54.14 ± 1.37	58.61 ± 0.73
pH 7.4 (Control)	28.88 ± 1.36	41.81 ± 1.66	36.91 ± 1.73	52.12 ± 1.86	56.81 ± 1.24	68.22 ± 0.91
pH 8	26.84 ± 1.03	36.75 ± 1.48	43.55± 1.19	59.61 ± 1.09	63.36 ± 1.46	79.32 ± 1.37
pH 9	26.17 ± 1.32	32.71 ± 1.36	34.72± 0.97	54.77 ± 1.15	57.66 ± 0.29	73.61 ± 1.33

Values represent mean of three replicates ± S.D.

Table 9. Effect of pH on the protein content (μg. ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	14.92 ± 0.27	14.98 ± 0.28	15.27 ± 013	15.34 ± 0.18	16.71 ± 0.36	17.02 ± 0.14
pH 4	15.16 ± 0.36	15.73 ± 0.31	16.25 ± 0.25	17.37 ± 0.52	18.21 ± 0.31	19.13 ± 0.21
pH 5	17.70 ± 0.45	17.92 ± 0.22	18.34 ± 0.36	18.73 ± 0.31	20.93 ± 0.27	21.87 ± 0.27
pH 6	22.91 ± 0.16	22.98 ± 0.19	23.17 ± 0.34	23.92 ± 0.23	25.03 ± 0.12	26.04 ± 0.23
pH 7	23.95 ± 0.23	28.73 ± 0.11	32.15 ± 0.57	36.45 ± 0.27	39.34 ± 0.19	40.16 ± 0.35
pH 7.4 (Control)	27.29 ± 0.52	32.46 ± 0.15	37.73 ± 0.41	42.70 ± 0.42	45.67 ± 0.16	46.78 ± 0.19
pH 8	25.33 ± 0.19	29.35 ± 0.27	36.16 ± 0.47	41.62 ± 0.51	43.26 ± 0.21	43.97 ± 0.13
pH 9	15.62 ± 0.33	18.01 ± 0.38	25.37 ± 0.24	32.76 ± 0.22	35.19 ± 0.27	39.01 ± 0.26

Values represent mean of three replicates ± S.D.

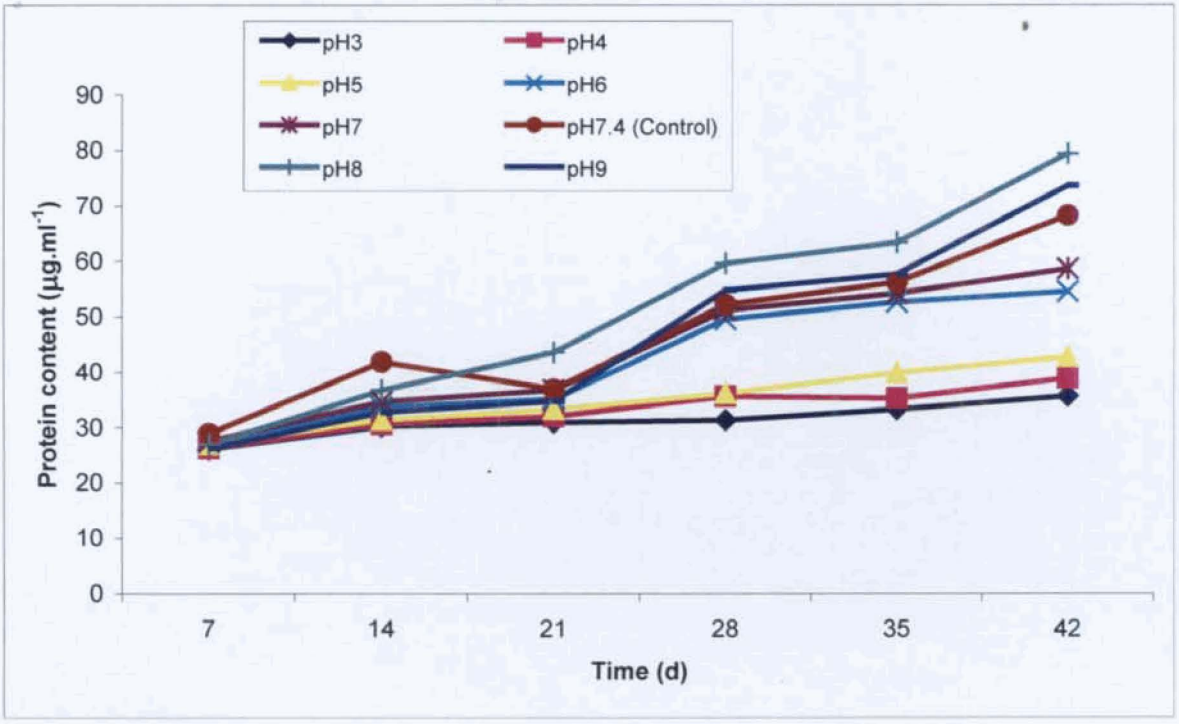


Fig. 8 Effect of pH on protein content of *Westiellopsis prolifica* up to 42 days of incubation.

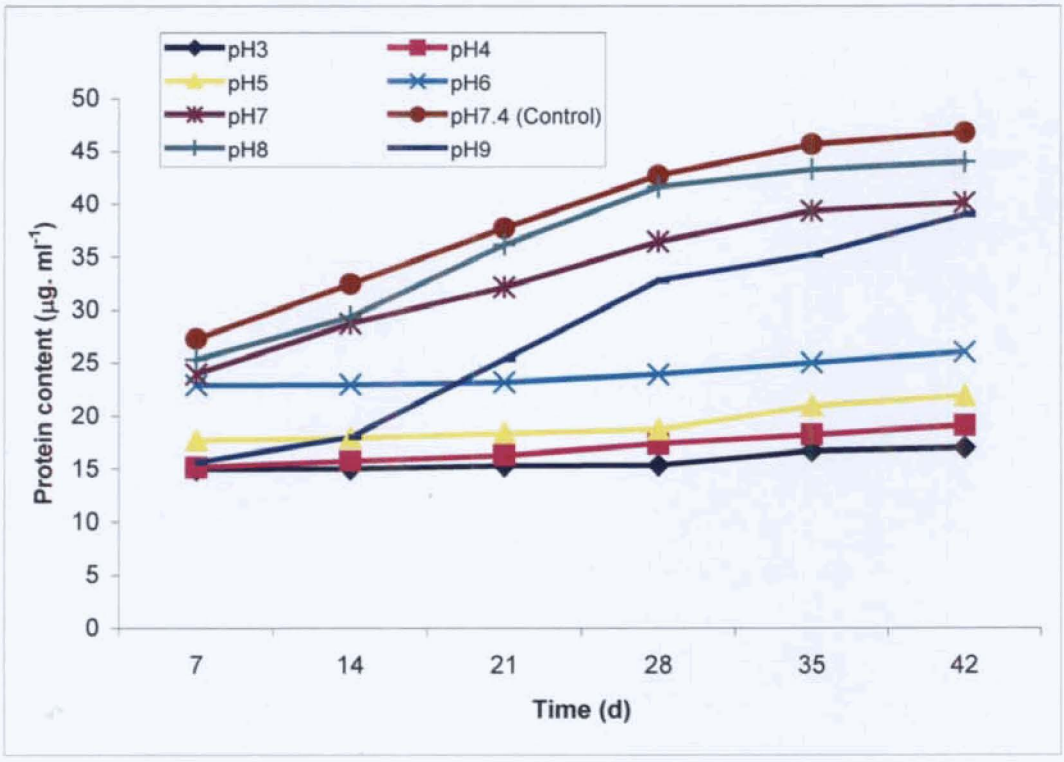


Fig. 9 Effect of pH on protein content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 10. Effect of pH on the chlorophyll-a content (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	0.93 ± 0.02	0.95 ± 0.13	0.98 ± 0.06	1.03 ± 0.14	1.32 ± 0.23	1.73 ± 0.11
pH 4	0.94 ± 0.01	0.98 ± 0.03	1.09 ± 0.09	1.19 ± 0.11	1.57 ± 0.19	1.91 ± 0.06
pH 5	0.96 ± 0.05	0.99 ± 0.08	1.32 ± 0.05	1.41 ± 0.13	1.82 ± 0.16	2.17 ± 0.09
pH 6	0.98 ± 0.03	1.25 ± 0.03	1.59 ± 0.07	1.63 ± 0.21	1.97 ± 0.13	2.86 ± 0.06
pH 7	0.99 ± 0.08	1.65 ± 0.13	2.01 ± 0.11	2.15± 0.17	2.72 ± 0.05	3.09 ± 0.01
pH 7.4 (Control)	1.01 ± 0.21	1.71 ± 0.08	2.95 ± 0.15	3.27 ± 0.08	3.92 ± 0.09	4.23 ± 0.05
pH 8	0.99 ± 0.12	1.79 ± 0.06	3.11 ± 0.17	3.92 ± 0.07	4.19 ± 0.03	4.99 ± 0.09
pH 9	0.96 ± 0.03	1.62 ± 0.01	2.99 ± 0.06	3.29 ± 0.09	3.86 ± 0.03	3.76 ± 0.13

Values represent mean of three replicates ± S.D.

Table 11. Effect of pH on the chlorophyll-a content (μg. ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	0.801 ± 0.09	0.804 ± 0.03	0.805 ± 0.11	0.808 ± 0.13	0.815 ± 0.07	0.819 ± 0.08
pH 4	0.835 ± 0.1	0.839 ± 0.05	0.841 ± 0.07	0.842 ± 0.15	0.844 ± 0.08	0.847 ± 0.13
pH 5	0.852 ± 0.23	0.853 ± 0.09	0.855 ± 0.06	0.858 ± 0.09	0.859 ± 0.12	0.860 ± 0.14
pH 6	0.897 ± 0.08	0.899 ± 0.12	0.92 ± 0.05	0.922 ± 0.11	0.928 ± 0.11	0.931 ± 0.17
pH 7	0.993 ± 0.06	1.071 ± 0.15	1.083 ± 0.04	1.097 ± 0.06	2.012 ± 0.19	2.093± 0.09
pH 7.4 (Control)	1.026 ± 0.13	1.082 ± 0.20	2.231 ± 0.17	2.662 ± 0.12	3.373 ± 0.18	3.734 ± 0.05
pH 8	1.012 ± 0.20	2.031 ± 0.19	2.132 ± 0.16	2.176 ± 0.14	3.127± 0.15	3.480 ± 0.04
pH 9	0.837 ± 0.11	1.832 ± 0.10	1.873 ± 0.21	2.036 ± 0.11	2.672 ± 0.16	2.983 ± 0.17

Values represent mean of three replicates ± S.D.

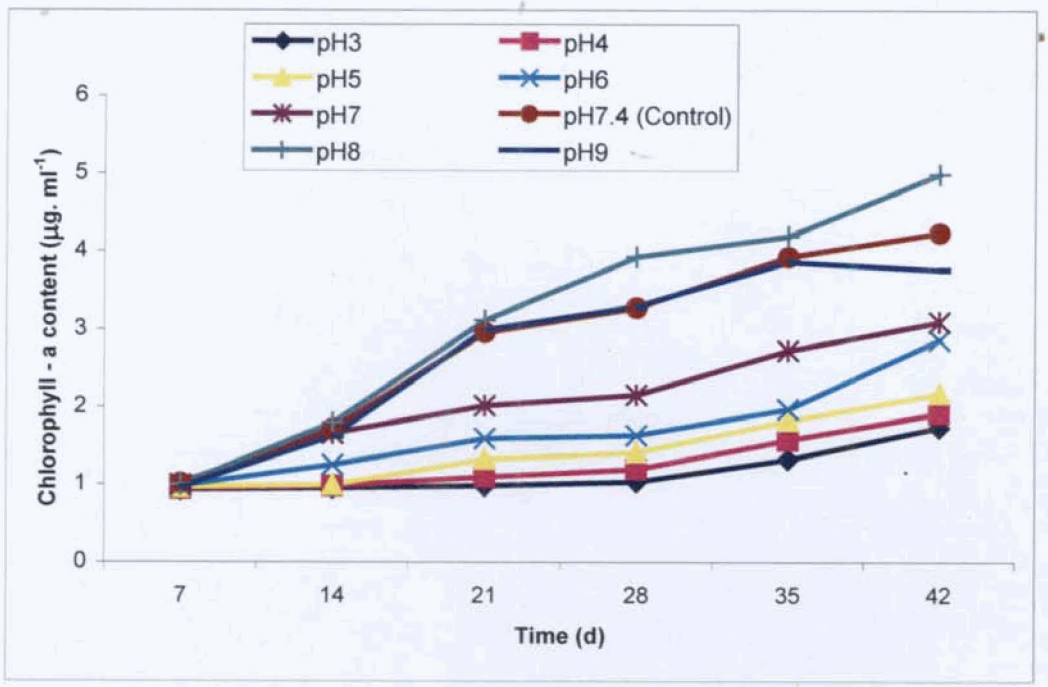


Fig.10 Effect of pH on chlorophyll-a content of *Westiellopsis prolifica* up to 42 days of incubation.

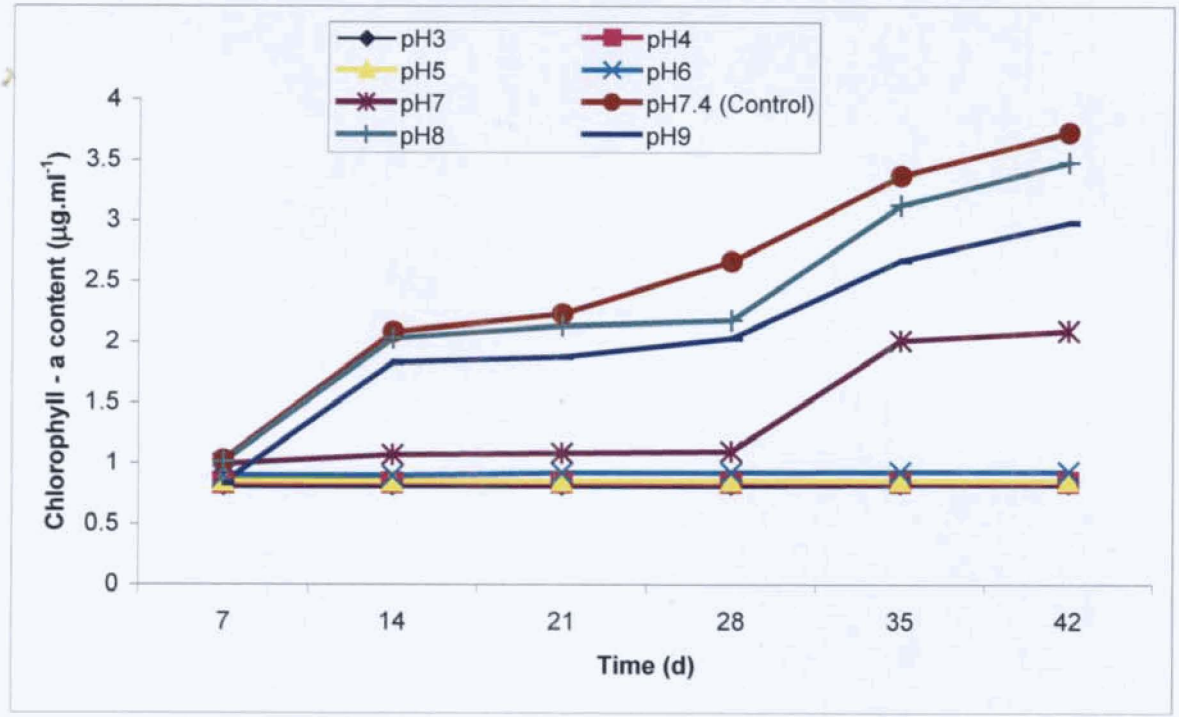


Fig.11 Effect of pH on chlorophyll-a content of *scytonema cincinnatum* up to 42 days of incubation.

Table 12. Effect of pH on the carotene content (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	0.149 ± 0.05	0.416 ± 0.09	0.531 ± 0.01	0.545 ± 0.06	0.612 ± 0.08	0.619 ± 0.07
pH 4	0.152 ± 0.05	0.423 ± 0.09	0.552± 0.11	0.563 ± 0.07	0.592 ± 0.09	0.598 ± 0.06
pH 5	0.179 ± 0.07	0.588 ± 0.08	0.660 ± 0.15	0.666 ± 0.07	0.677 ± 0.01	0.693 ± 0.09
pH 6	0.190 ± 0.06	0.655 ± 0.06	0.672 ± 0.02	0.677 ± 0.13	0.694 ± 0.03	0.705 ± 0.16
pH 7	0.235 ± 0.02	0.672 ± 0.01	0.683 ±0.06	0.711 ± 0.15	0.722 ± 0.02	0.784 ± 0.11
pH 7.4 (Control)	0.296 ± 0.02	0.688 ± 0.02	0.694 ± 0.04	0.739 ± 0.19	0.756 ± 0.02	0.884 ± 0.04
pH 8	0.268 ± 0.01	0.692 ± 0.03	0.700 ± 0.07	0.845 ± 0.06	0.873 ± 0.09	0.918 ± 0.06
pH 9	0.196 ± 0.03	0.644 ± 0.03	0.673 ± 0.09	0.728 ± 0.08	0.736 ± 0.07	0.784 ± 0.09

Values represent mean of three replicates ± S.D.

Table 13. Effect of pH on the carotene content (μg. ml^{-1} culture) of *Scytonema cincinnatum* upto 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	0.072 ± 0.05	0.073 ± 0.16	0.074 ± 0.07	0.079 ± 0.02	0.081 ± 0.12	0.081 ± 0.12
pH 4	0.083 ± 0.11	0.087 ± 0.03	0.093± 0.05	0.094 ± 0.09	0.094 ± 0.07	0.095 ± 0.11
pH 5	0.091 ± 0.17	0.106 ± 0.08	0.107 ± 0.06	0.112 ± 0.09	0.116 ± 0.08	0.119 ± 0.16
pH 6	0.113 ± 0.06	0.115 ± 0.02	0.117 ± 0.02	0.121 ± 0.11	0.123 ± 0.05	0.124 ± 0.14
pH 7	0.141 ± 0.07	0.153 ± 0.07	0.157 ± 0.04	0.162 ± 0.13	0.287 ± 0.04	0.291 ± 0.08
pH 7.4 (Control)	0.146 ± 0.13	0.154 ± 0.13	0.175 ± 0.11	0.237 ± 0.17	0.310 ± 0.13	0.312 ± 0.06
pH 8	0.126 ± 0.08	0.128 ± 0.15	0.141 ± 0.08	0.147 ± 0.19	0.152 ± 0.05	0.156 ± 0.13
pH 9	0.093 ± 0.11	0.094 ± 0.09	0.109 ± 0.12	0.117 ± 0.16	0.121 ± 0.09	0.125 ± 0.12

Values represent mean of three replicates ± S.D.

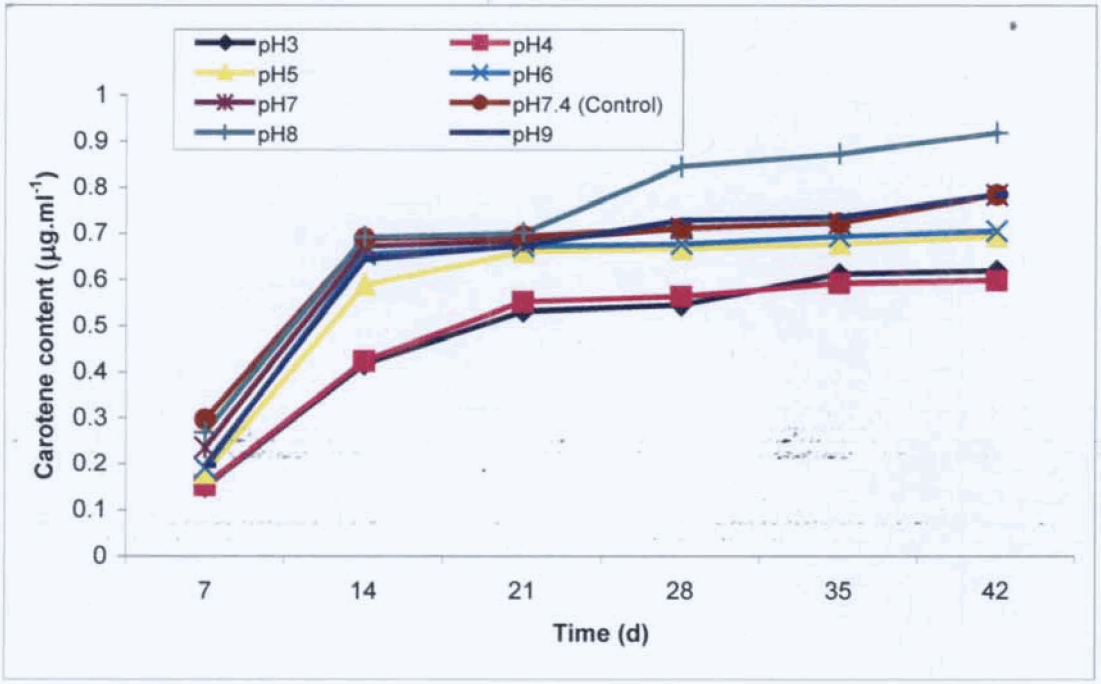


Fig.12 Effect of pH on carotenoid content of *Westiellopsis prolifica* up to 42 days of incubation.

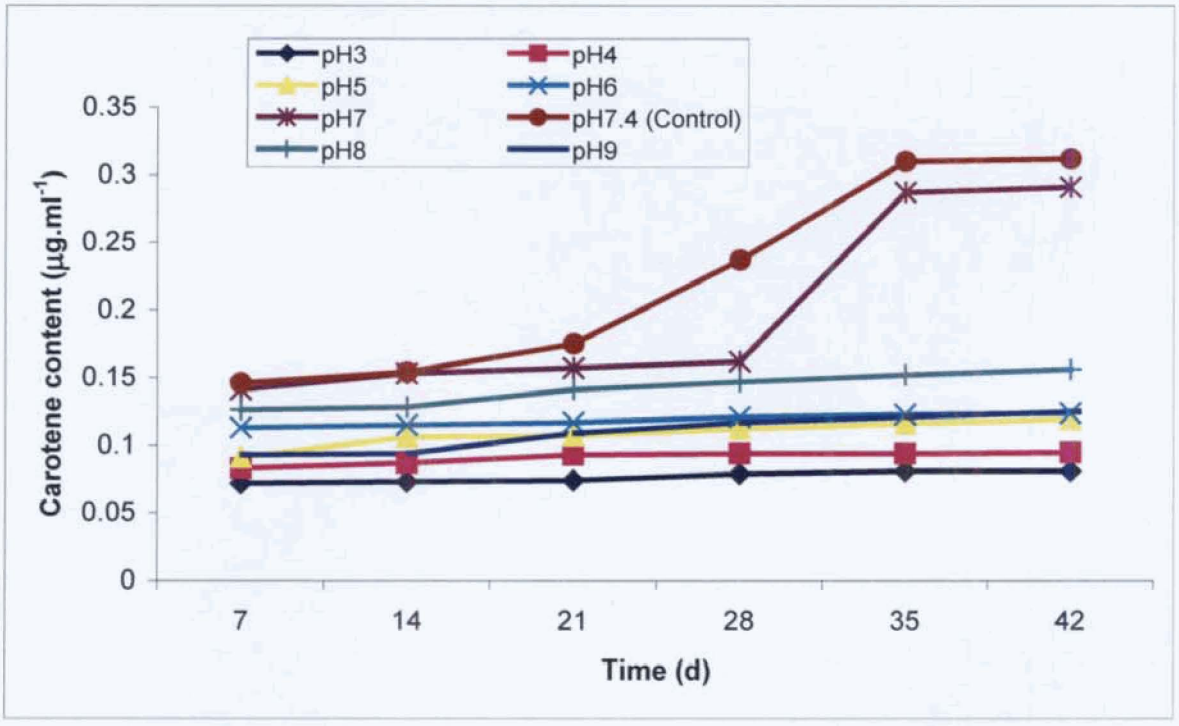


Fig.13 Effect of pH on carotenoid content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 14. Effect of pH on phycobilin pigment C-phycocyanin (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	7.16 ± 0.06	7.92 ± 0.09	8.01 ± 0.07	10.26 ± 0.07	11.06 ± 0.11	12.56 ± 0.07
pH 4	7.19 ± 0.05	8.23 ± 0.06	9.13 ± 0.02	14.13 ± 0.03	16.03 ± 0.09	18.62 ± 0.03
pH 5	7.22 ± 0.09	10.22 ± 0.06	12.61 ± 0.03	15.4 ± 0.01	17.61 ± 0.09	19.1 ± 0.12
pH 6	7.43 ± 0.06	16.43 ± 0.05	13.60 ± 0.06	18.16 ± 0.02	19.51 ± 0.06	20.32 ± 0.01
pH 7	9.48 ± 0.12	16.81 ± 0.01	17.62 ± 0.07	18.75 ± 0.09	19.58 ± 0.01	21.16 ± 0.08
pH 7.4 (Control)	12.12 ± 0.11	20.6 ± 0.02	20.72 ± 0.01	22.14 ± 0.11	25.84 ± 0.03	26.71 ± 0.03
pH 8	7.61 ± 0.07	18.41 ± 0.02	20.57 ± 0.02	23.19 ± 0.13	25.97 ± 0.05	27.83 ± 0.07
pH 9	5.22 ± 0.08	14.71 ± 0.07	13.41 ± 0.03	18.76 ± 0.10	19.73 ± 0.02	19.92 ± 0.06

Values represent mean of three replicates ± S.D.

Table 15. Effect of pH on phycobilin pigment allophycocyanin (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	8.16 ± 0.21	9.23 ± 0.17	12.36 ± 0.01	13.72 ± 0.19	15.61 ± 0.11	17.13 ± 0.07
pH 4	8.21 ± 0.23	9.29 ± 0.16	15.29 ± 0.06	17.23 ± 0.16	19.92 ± 0.07	20.61 ± 0.09
pH 5	8.26 ± 0.16	10.81 ± 0.05	19.23 ± 0.11	20.41 ± 0.05	26.41 ± 0.089	27.13 ± 0.11
pH 6	8.61 ± 0.09	16.42 ± 0.28	22.62 ± 0.12	23.86 ± 0.06	30.88 ± 0.09	31.32 ± 0.16
pH 7	9.12 ± 0.11	26.40 ± 0.13	26.68 ± 0.06	24.42 ± 0.07	33.86 ± 0.05	35.61 ± 0.09
pH 7.4 (Control)	15.11 ± 0.13	29.41 ± 0.06	29.69 ± 0.05	31.81 ± 0.08	34.89 ± 0.03	36.72 ± 0.07
pH 8	15.21 ± 0.08	25.60 ± 0.08	31.41 ± 0.21	32.27 ± 0.09	35.11 ± 0.09	38.16 ± 0.13
pH 9	9.41 ± 0.22	21.44 ± 0.07	28.42 ± 0.26	29.16 ± 0.06	31.62 ± 0.13	32.12 ± 0.15

Values represent mean of three replicates ± S.D.

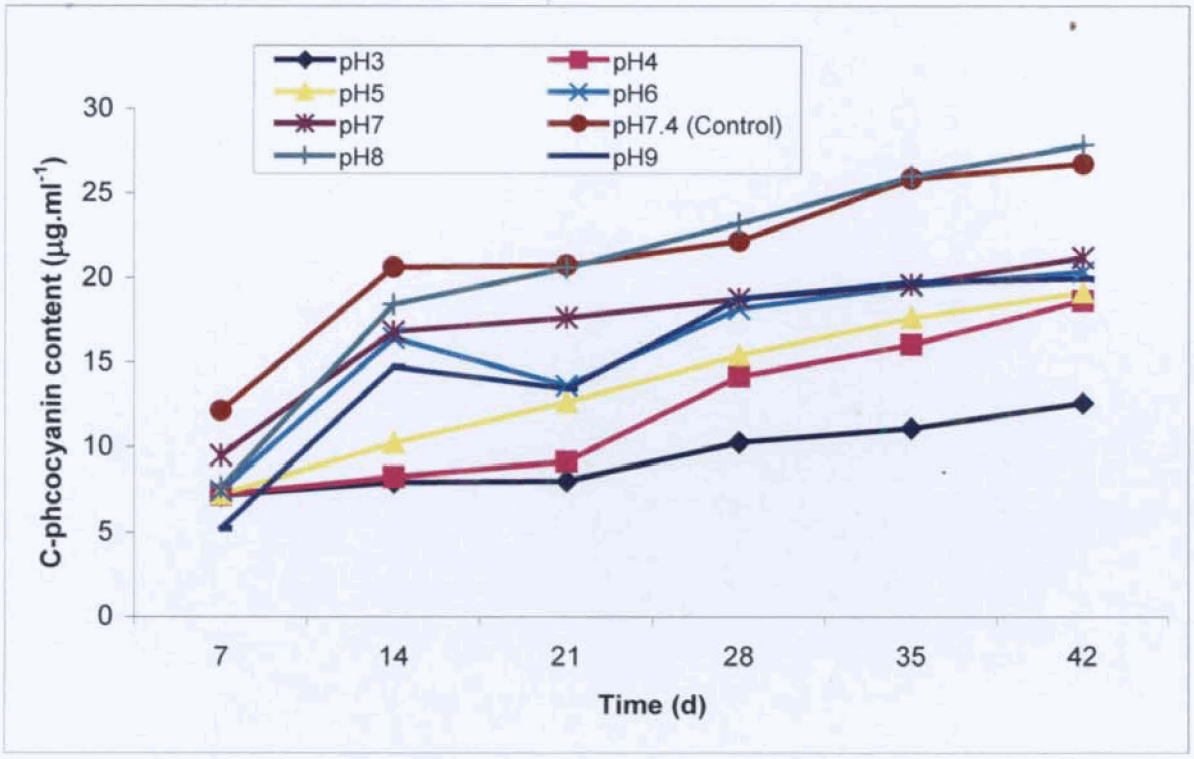


Fig.14 Effect of pH on c-phycocyanin content of *Westiellopsis prolifica* up to 42 days of incubation.

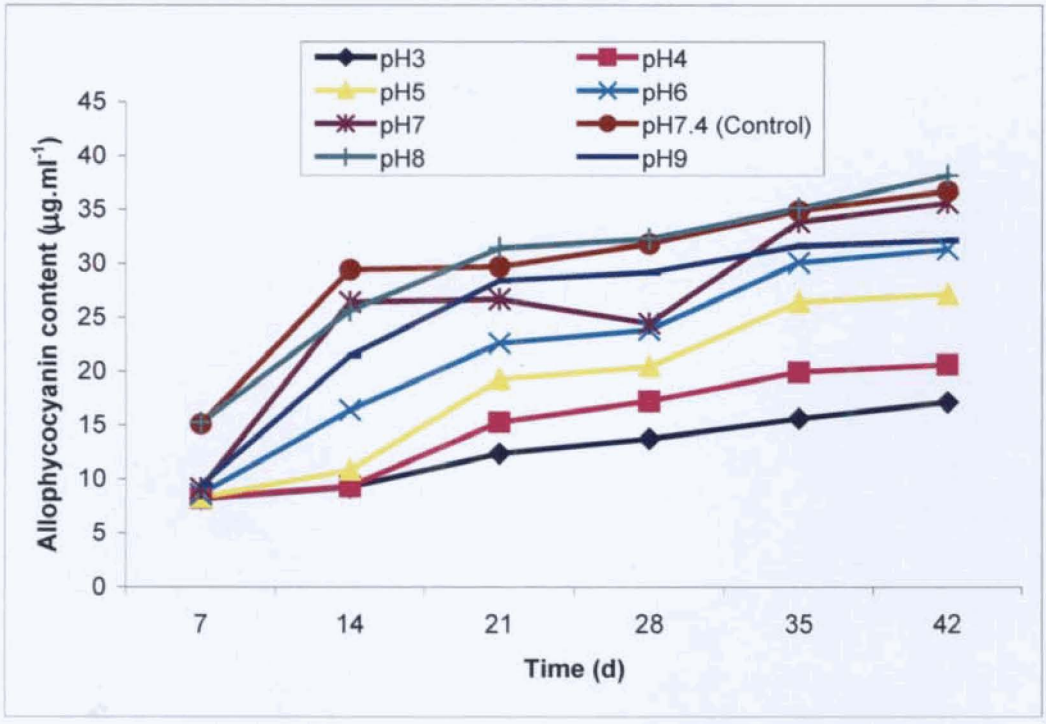


Fig.15 Effect of pH on allophycocyanin content of *Westiellopsis prolifica* up to 42 days of incubation.

Table 16. Effect of pH on the phycobilin pigment C–phycoerythrin (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	1.01 ± 0.06	1.11 ± 0.07	1.19 ± 0.11	1.28 ± 0.05	1.36 ± 0.17	1.42 ± 0.11
pH 4	1.09 ± 0.09	1.15 ± 0.17	1.23 ± 0.05	1.36 ± 0.07	1.52 ± 0.09	1.93 ± 0.23
pH 5	1.12 ± 0.08	1.19 ± 0.09	1.31 ± 0.07	1.45 ± 0.18	1.97 ± 0.02	2.11 ± 0.19
pH 6	1.16 ± 0.15	1.24 ± 0.08	1.38 ± 0.13	1.49 ± 0.15	2.01 ± 0.11	2.19 ± 0.12
pH 7	1.23 ± 0.16	1.36 ± 0.16	1.54± 0.19	1.58 ± 0.11	2.23 ± 0.16	2.52± 0.09
pH 7.4 (Control)	1.29 ± 0.07	1.52 ± 0.11	1.62 ± 0.17	1.79 ± 0.23	2.45 ± 0.19	2.65 ± 0.17
pH 8	1.27 ± 0.03	1.59 ± 0.19	1.89 ± 0.11	1.95 ± 0.21	2.57 ± 0.20	2.93 ± 0.12
pH 9	1.18 ± 0.02	1.47 ± 0.11	1.53 ± 0.05	1.67 ± 0.09	2.41 ± 0.17	2.42 ± 0.08

Values represent mean of three replicates ± S.D.

Table 17. Effect of pH on the phycobilin pigment – c-phycocyanin (μg. ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	14.11 ± 0.11	14.13 ± 0.17	15.09 ± 0.12	15.11 ± 0.13	15.57 ± 0.06	15.67 ± 0.16
pH 4	14.87 ± 0.15	15.07 ± 0.11	15.24 ± 0.13	15.78 ± 0.11	15.87 ± 0.07	16.36 ± 0.12
pH 5	15.70 ± 0.09	15.61 ± 0.15	15.55 ± 0.15	16.24 ± 0.08	16.54 ± 0.11	17.29 ± 0.13
pH 6	17.39 ± 0.15	17.18 ± 0.08	17.47 ± 0.09	17.97 ± 0.08	18.06 ± 0.13	18.41 ± 0.03
pH 7	17.72 ± 0.06	18.97 ± 0.09	20.37 ± 0.11	21.04 ± 0.09	22.69 ± 0.15	23.38 ± 0.05
PH 7.4 (Control)	19.62 ± 0.12	20.48 ± 0.11	21.79 ± 0.07	23.77 ± 0.13	25.22 ± 0.14	25.49 ± 0.13
pH 8	18.17 ± 0.13	19.71 ± 0.17	21.31 ± 0.06	23.01 ± 0.10	24.42 ± 0.15	24.65 ± 0.11
pH 9	15.03 ± 0.13	15.62 ± 0.07	17.68 ± 0.12	20.92 ± 0.11	21.73 ± 0.09	23.02 ± 0.08

Values represent mean of three replicates ± S.D.

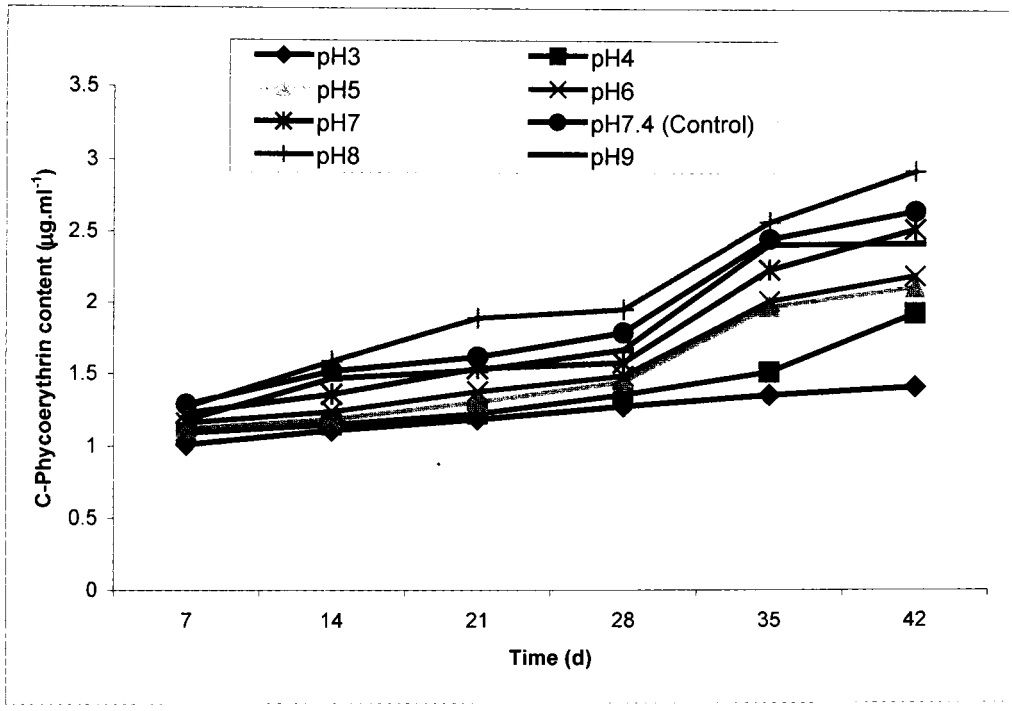


Fig.16 Effect of pH on c-phycoerythrin content of *Westiellopsis prolifica* up to 42 days of incubation.

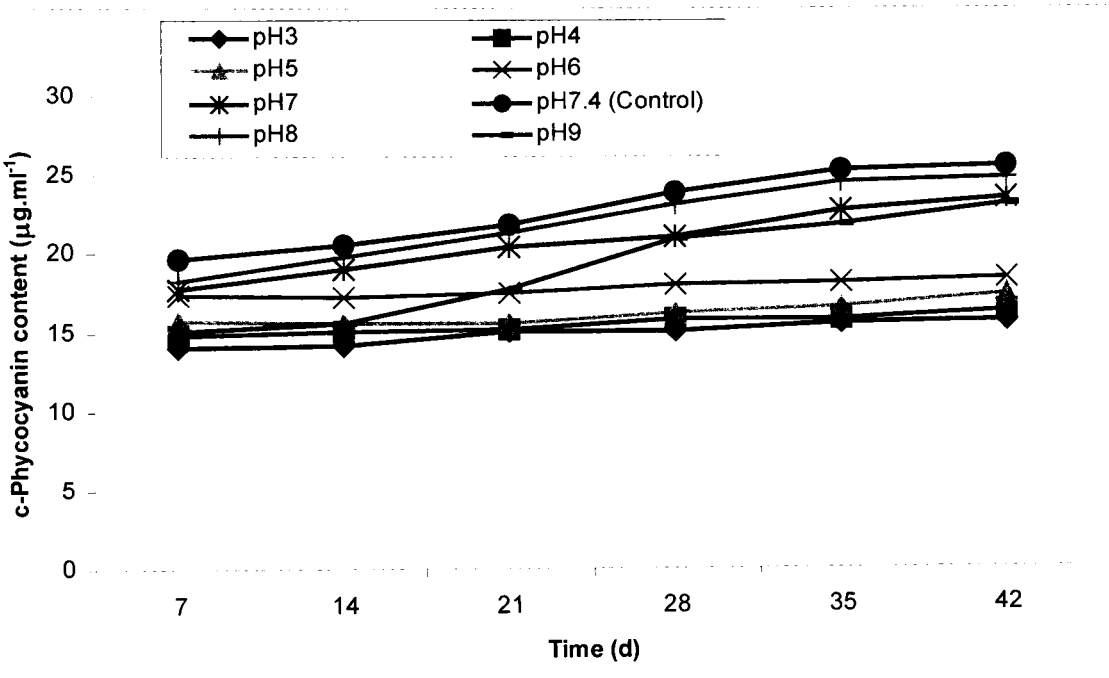


Fig.17 Effect of pH on c-phycocyanin content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 18. Effect of pH on the phycobilin pigment allophycocyanin (μg. ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	12.92 ± 0.07	12.97 ± 0.21	13.51 ± 0.10	13.73 ± 0.31	13.92 ± 0.12	14.01 ± 0.19
pH 4	13.17 ± 0.17	13.73 ± 0.23	13.86 ± 0.09	14.01 ± 0.16	14.32 ± 0.14	14.76 ± 0.21
pH 5	14.12 ± 0.16	14.57 ± 0.16	14.83 ± 0.13	15.73 ± 0.19	15.87 ± 0.09	16.05 ± 0.08
pH 6	15.91 ± 0.09	16.02 ± 0.09	16.35 ± 0.21	16.79 ± 0.08	17.21 ± 0.11	17.62 ± 0.09
pH 7	16.17 ± 0.11	17.23 ± 0.14	18.37 ± 0.23	19.03 ± 0.13	20.45 ± 0.07	21.71 ± 0.13
pH 7.4 (Control)	17.98 ± 0.23	18.53 ± 0.19	18.76 ± 0.07	19.37 ± 0.17	21.47 ± 0.09	22.67 ± 0.11
pH 8	16.54 ± 0.17	17.27 ± 0.14	17.81 ± 0.19	18.79 ± 0.18	19.11 ± 0.12	20.62 ± 0.07
pH 9	15.37 ± 0.21	14.12 ± 0.11	16.37 ± 0.18	17.36 ± 0.11	18.07 ± 0.12	19.17 ± 0.13

Values represent mean of three replicates ± S.D.

Table 19. Effect of pH on the phycobilin pigment c-phycoerythrin (μg. ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	1.576 ± 0.011	1.582 ± 0.06	1.593 ± 0.07	1.597 ± 0.02	1.602 ± 0.09	1.612 ± 0.06
pH 4	1.584 ± 0.03	1.596 ± 0.07	1.605 ± 0.08	1.611 ± 0.05	1.612 ± 0.03	1.615 ± 0.07
pH 5	1.596 ± 0.09	1.613 ± 0.02	1.614± 0.11	1.621 ± 0.06	1.629 ± 0.05	1.533 ± 0.07
pH 6	1.607 ± 0.03	1.615 ± 0.03	1.619 ± 0.12	1.628 ± 0.05	1.636 ± 0.03	1.640 ± 0.09
pH 7	1.618 ± 0.05	1.624 ± 0.02	1.633 ± 0.09	1.642 ± 0.03	1.659 ± 0.04	1.675 ± 0.05
pH 7.4 (Control)	1.627 ± 0.02	1.635 ± 0.05	1.651 ± 0.05	1.663 ± 0.10	1.682 ± 0.05	1.695 ± 0.03
pH 8	1.621 ± 0.11	1.629 ± 0.04	1.635 ± 0.03	1.657 ± 0.07	1.668 ± 0.09	1.681 ± 0.08
pH 9	1.617 ± 0.02	1.625 ± 0.01	1.628 ± 0.06	1.629 ± 0.08	1.631 ± 0.05	1.635 ± 0.06

Values represent mean of three replicates ± S.D.

Table 20. Effect of pH on ammonia excretion (n moles ml^{-1}) by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation

pH	3	4	5	6	7	7.4 (Control)	8	9
W. prolifica	20.26 ± 0.09	21.33 ± 0.10	23.21 ± 0.08	25.26 ± 0.09	25.67 ± 0.06	27.14 ± 0.07	29.11 ± 0.09	28.13 ± 0.08
S. cincinnatum	14.07 ± 0.04	15.38 ± 0.07	14.74 ± 0.05	15.94 ± 0.08	18.62 ± 0.07	20.37 ± 0.03	19.42 ± 0.08	18.92 ± 0.07

Values represent mean of three replicates ± S.D.

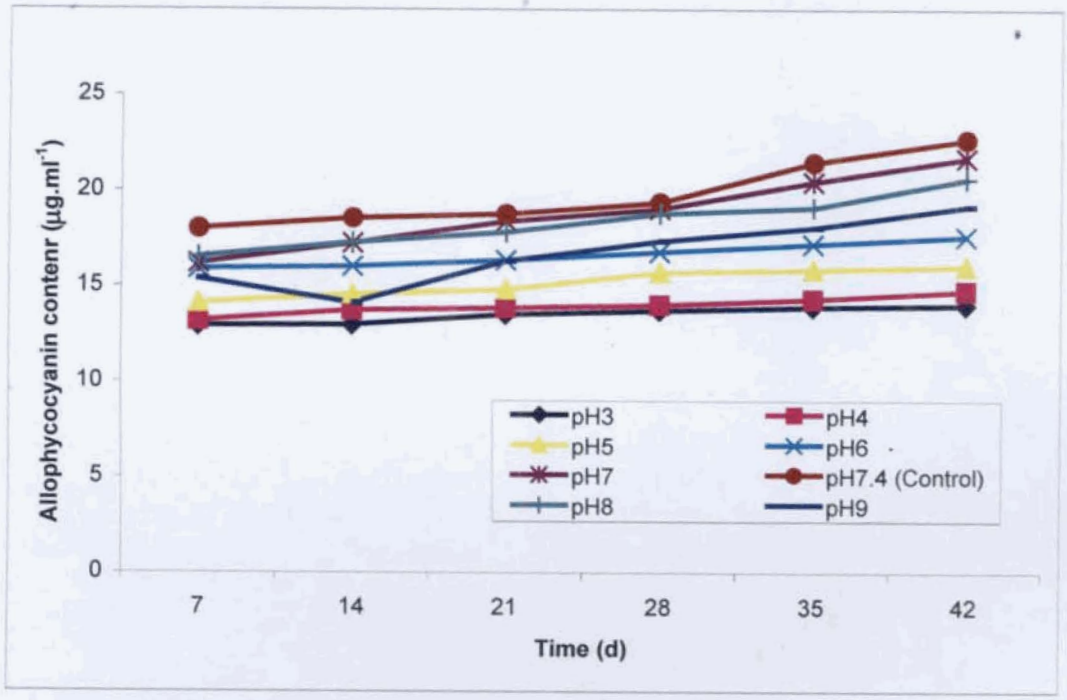


Fig.18 Effect of pH on allophycocyanin content of *Scytonema cincinnatum* up to 42 days of incubation.

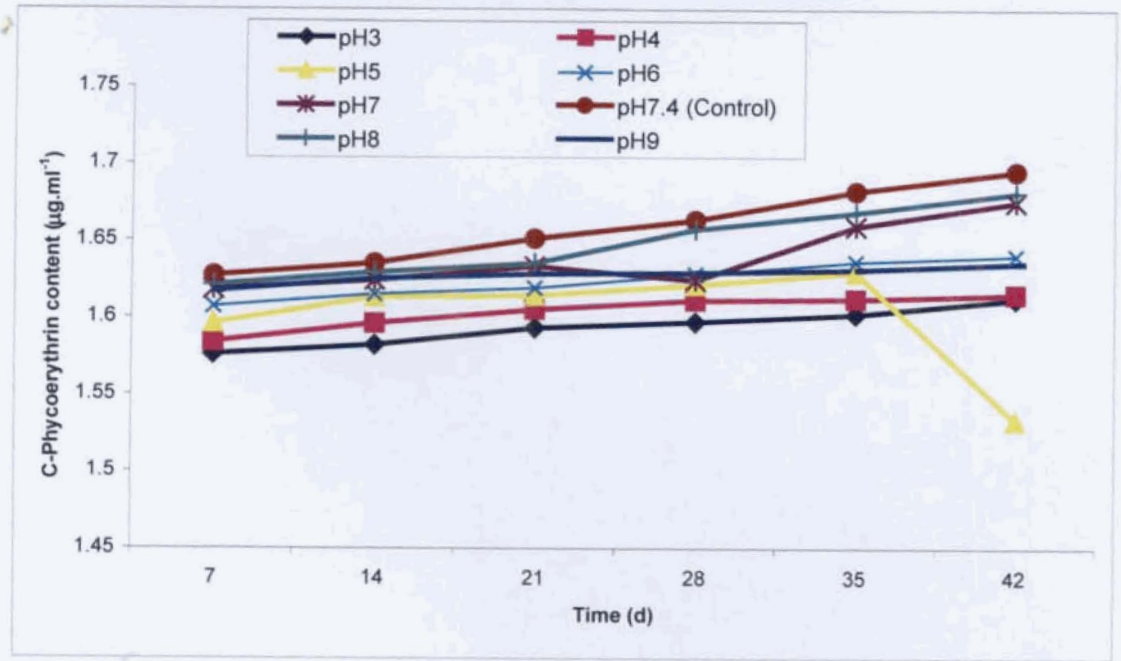


Fig.19 Effect of pH on c-phycoerythrin content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 21. Heterocyst frequency (%) of *Westiellopsis prolifica* at different levels of pH

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	4.6 ± 0.16	4.9 ± 0.26	5.4 ± 0.27	5.7 ± 0.11	6.2 ± 0.19	6.6 ± 0.29
pH 4	4.9 ± 0.21	5.2 ± 0.33	5.9 ± 0.23	5.9 ± 0.16	7.1 ± 0.34	7.5 ± 0.28
pH 5	4.3 ± 0.23	5.3 ± 0.21	6.3 ± 0.21	6.6 ± 0.24	7.4 ± 0.14	7.9 ± 0.17
pH 6	5.3 ± 0.25	6.3 ± 0.14	8.2 ± 0.29	9.1 ± 0.36	9.3 ± 0.19	9.8 ± 0.23
pH 7	5.9 ± 0.22	7.6 ± 0.63	10.1 ± 0.32	11.3 ± 0.21	11.6 ± 0.26	11.9 ± 0.16
pH 7.4 (Control)	6.6± 0.26	8.3 ± 0.59	11.3 ± 0.17	11.5 ±0.21	12.1 ± 0.32	12.4 ± 0.17
pH 8	7.6 ± 0.29	9.8 ± 0.16	11.9 ± 0.21	12.5 ± 0.26	13.3 ± 0.16	13.9 ± 0.26
pH 9	7.5 ± 0.21	9.2 ± 0.23	10.7 ± 0.53	12.1 ± 0.19	12.5 ± 0.11	12.6 ± 0.19

Values represent mean of three replicates ± S.D.

Table 22. Heterocysts frequency of *Scytonema cinncinatum* at different pH levels

	Days after inoculation					
pH	7	14	21	28	35	42
pH3	4.1 ± 0.19	4.2 ± 0.16	4.5 ± 0.19	4.8 ± 0.16	5.1 ± 0.15	5.7 ± 0.16
pH 4	4.1 ± 0.23	4.5 ± 0.21	4.7 ± 0.23	4.8 ± 0.21	5.2 ± 0.22	5.7 ± 0.19
pH 5	4.5 ± 0.21	4.6 ± 0.24	4.9 ± 0.26	5.3 ± 0.22	5.9 ± 0.23	6.3 ± 0.21
pH 6	4.9 ± 0.22	5.3 ± 0.13	5.6 ± 0.14	5.8 ± 0.19	6.2 ± 0.15	6.7 ± 0.14
pH 7	5.3 ± 0.18	5.5 ± 0.21	5.7 ± 0.14	6.1 ± 0.13	6.5 ± 0.14	7.1 ± 0.15
pH 7.4 (Control)	6.8 ± 0.15	6.5 ± 0.26	7.2 ± 0.21	7.5 ± 0.22	8.1 ± 0.21	8.4 ± 0.21
pH 8	6.4 ± 0.14	6.7 ± 0.23	6.9 ± 0.19	7.1 ± 0.18	7.9 ± 0.16	8.9 ± 0.17
pH 9	5.5 ± 0.21	5.9 ± 0.24	6.1± 0.21	6.3 ± 0.22	6.9 ± 0.19	7.2 ± 0.18

Values represent mean of three replicates ± S.D.

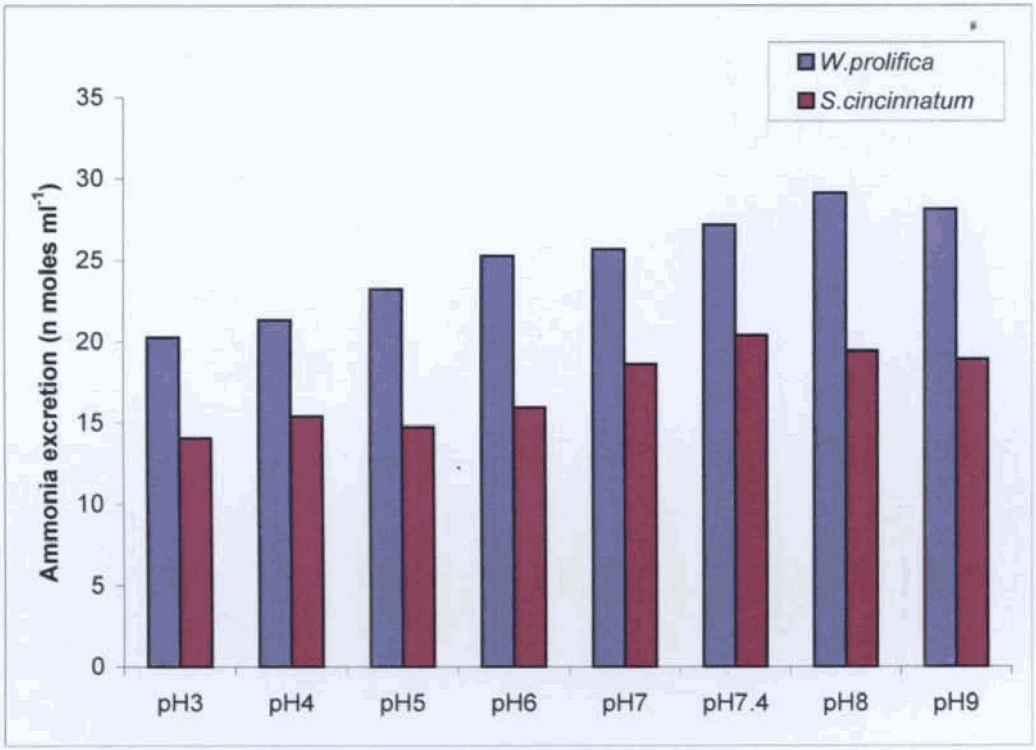


Fig.20 Effect of pH on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation.

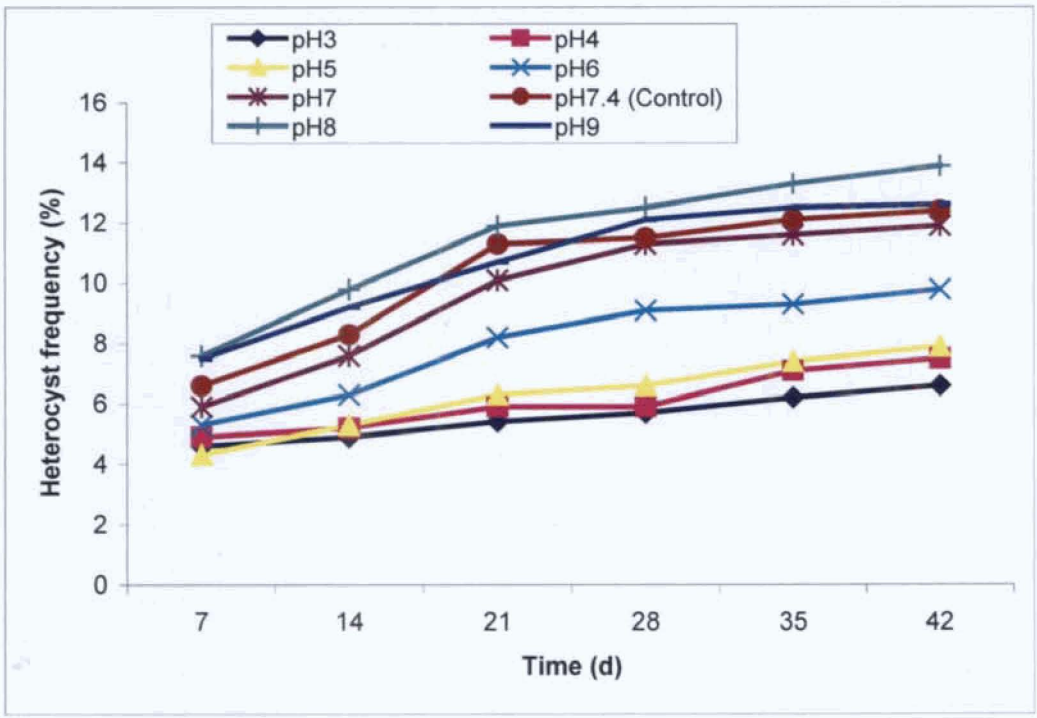


Fig.21 Effect of pH on heterocyst abundance of *Westiellopsis prolifica* up to 42 days of incubation.

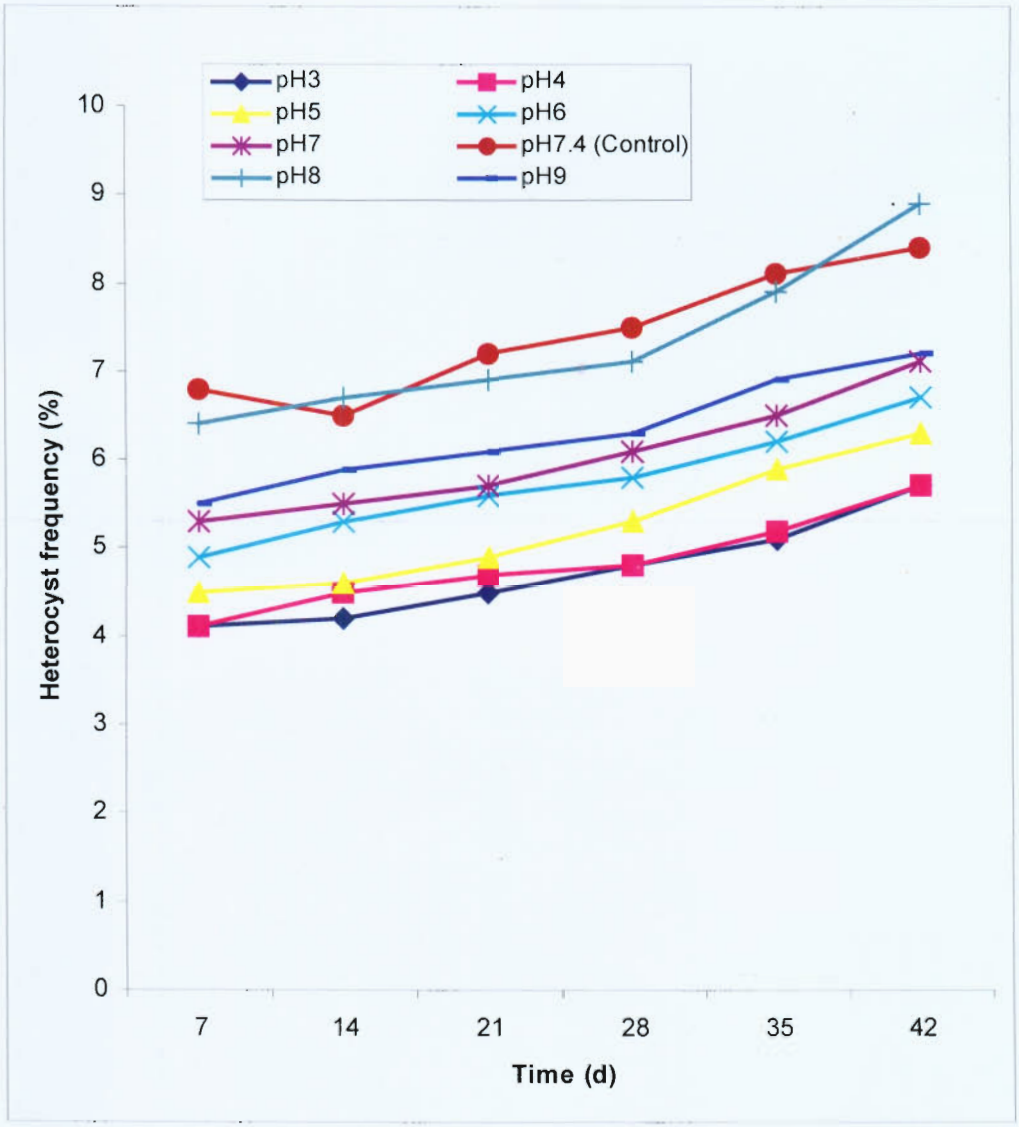


Fig.22 Effect of pH on heterocyst abundance of *Scytonema cincinnatum* up to 42 days of incubation.

Morphological studies on *Westiellopsis prolifica* revealed that at acidic ranges of pH, the vegetative cells as well as heterocysts became short, 5-6 μm in length, heterocyst and branching of the filaments were not frequent and the length of the branch was also short. But in neutral to alkaline ranges of pH, the vegetative cells and heterocysts became enlarged to 7.5 – 15.0 μm in length, heterocyst and branching frequency increased and was maximum at pH 8. While in *Scytonema cincinnatum* there was no reduction in the size of vegetative cells and heterocysts from acidic to alkaline range while the branching of filaments was maximum at alkaline ranges of pH.

4.2.2. Discussion

Analysis of the data showed that both the species of cyanobacteria can tolerate a wide pH range though they grew well and fixed more N_2 in the alkaline range. Previous studies have shown that among the several soil properties, pH is the most important factor determining the composition of cyanobacterial flora in natural ecosystems. Positive correlation between pH and the occurrence, abundance or growth of cyanobacteria were reported by several authors (Roger and Reynaud, 1977; Roger *et al.*, 1987).

In the present study, it is found that both the cyanobacteria can grow in wide range of pH. The fairly good growth at pH 3.0 to 6.0 shows that *Westiellopsis prolifica* and *Scytonema cincinnatum* can thrive well in acidic soils also and it agrees with the findings of Tamilselvam *et al.* (2001). The present study reveals that initially for 15-20 days, the growth of *Westiellopsis*

prolifica was low but showed remarkable increase at pH 8.0 and 9.0 with maximum at pH 8. While *Scytonema cincinnatum* shows almost uniform growth up to the end of the experiment.

Westiellopsis prolifica took about 2-3 weeks to adjust to the pH of the growing medium. Increase in the concentration of protein and pigments according to increase in pH shows that there will be variation in protein content and pigment content when grown under different pH levels. Increased chlorophyll-a content at alkaline range is due to the increased photosynthetic activity of the cyanobacteria and the increased ammonia excretion at alkaline range indicates the increased N_2 fixation by the heterocysts. Thus, both cyanobacteria show a positive correlation between the growth and biochemical constituents at various pH levels.

Cyanobacteria generally prefer neutral to slightly alkaline pH for optimum growth (Rath and Adhikary, 1996). Acidic soils are therefore one of the stressed environments for these organisms and are normally rare at pH values 4 or 5; eukaryotic algae however, flourish under these conditions (Brock, 1973b; Fogg *et al.*, 1973). In culture media the optimal pH for cyanobacterial growth ranges from 7.5 to 10.0 and its lower limit was about 6.5 to 7.0 (Holm-Hansen, 1968). But there are reports on the occurrence of certain cyanobacteria in soils with pH between 5.0 and 6.0 (Prasad *et al.*, 1978) and lower than 5.0 (Aiyer, 1965; Roger *et al.*, 1987; Dominic and Madhusoodanan, 1999). *Aulosira fertilissima* and *Calothrix brevissima* have been found to be ubiquitous in Kerala rice fields having pH varying from 3.5

to 6.5 (Anand and Hopper, 1987, 1995; Madhusoodhanan and Dominic, 1995). Presence of *Nostoc muscorum* and *Anabaena torulosa* has been reported in soils with pH ranging from 5.0 to 7.0 (Durrel, 1964). A rapid growth of some strains under laboratory conditions in a medium of low pH has been reported and they maintained a high intracellular pH (Kallas and Castenholz, 1982). Singh *et al.* (1995) examined cyanobacterial isolates obtained from alkaline soils for their response to changes in pH. Most of the isolates liked higher pH while few of them preferred low pH or a wider pH range. pH of the habitat from which the cyanobacteria were isolated did not seem to confer pH specificity.

Thus the present study shows that *Westiellopsis prolifica* and *Scytonema cincinnatum* can thrive well in the acidic paddy fields of Kerala and elsewhere. Both these strains had a remarkable degree of tolerance to high levels of H^+ concentrations. However, strain specificity occurs among different genera for their optima for growth and nitrogen fixation. These cyanobacteria presumably have an efficient internal pH regulating mechanism (Fogg *et al.*, 1973). Maintenance of high internal pH in cells during growth limiting condition and lethal exposures to low external pH values suggest that low pH acts initially on cell membrane (Kallas and Castenholz, 1982). It also reveals that pH has a significant role in growth, branching frequency, production of heterocysts and excretion of ammonia. The capacity of cyanobacteria of releasing ammonia continuously into the environment is of potential use as biofertilizer in rice fields. Moreover, the

soil of paddy fields of Kerala is generally acidic except for a small area in Palakkad district. Thus the present results agree with the earlier observation (Madhusoodanan and Dominic, 1996) that it can occur in extreme acidic (pH 4.0 and 5.0) soils of Kerala state. Thus *Westiellopsis prolifica* and *Scytonema cincinnatum* has a great biofertilizer potential in Kerala state where most of the paddy fields are acidic.

4.3. GROWTH CHARACTERISTICS OF *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM* AT DIFFERENT CONCENTRATIONS OF NaCl

4.3.1. Results

The growth and biochemical characters of *Westiellopsis prolifica* and *Scytonema cincinnatum* were presented in the Tables (23-33). The cyanobacterium *Westiellopsis prolifica* showed considerable growth in BG-11 liquid medium amended with NaCl. This halotolerant cyanobacteria grow well up to 0.9 M NaCl with maximum growth at 0.6M NaCl (Table & Fig. 23). While *Scytonema cincinnatum*, a non halotolerant form grows well up to 0.8M NaCl for the first three weeks time interval with maximum growth at 0.4M NaCl. On the fourth week of incubation onwards the growth rate slows down and there will be no growth on 0.7, 0.8 and 0.9 M NaCl and the filaments loose its green colour and start to disintegrate (Table & Fig. 24). In *Westiellopsis prolifica* protein content was maximum at 0.6M NaCl and it showed tremendous increase after 35 days of incubation and the amount was double over the control (Table & Fig. 25). In *Scytonema cincinnatum*

the protein content was maximum for the control. On the 7th day of incubation the protein content was maximum at 0.4M NaCl. On the 14th day of incubation onwards the protein content began to decrease and 0.4M NaCl showed maximum protein content (Table & Fig. 26). Among pigments, chlorophyll-a and carotene content was maximum at 0.6M NaCl for *Westiellopsis prolifica* and the increase was less when concentration was higher (Table & Fig. 27, 29). While in *Scytonema cincinnatum* the maximum chlorophyll-a content was at 0.6M NaCl upto 14 days of incubation. On 21st day of incubation onwards the chlorophyll-a content decreased from 0.5M NaCl to 0.9M NaCl and the chlorophyll-a content was maximum at 0.4M NaCl. After one month of incubation there will be no chlorophyll content at 0.7, 0.8 and 0.9M NaCl, the chlorophyll-a content was maximum for the control and among different concentrations of NaCl 0.1M NaCl shows maximum chlorophyll content (Table & Fig. 28). On 42nd day of incubation the carotene content was maximum at 0.1M NaCl for *Scytonema cincinnatum* (Table & Fig. 30). Among phycobilin pigments, allophycocyanin release was more in *Westiellopsis prolifica* than phycocyanin and phycoerythrin content. *Westiellopsis prolifica* showed its maximum amount at 0.9M on the 42nd day of incubation and the amount of phycobilin content was lesser than the control (Table & Fig. 31). In *Scytonema cincinnatum* the release of pigments were in the order phycocyanin > allophycocyanin > phycoerythrin and it showed maximum release at 0.9M NaCl over the control (Table & Fig. 32). The ammonia release was maximum at 0.6M NaCl over

the control for *Westiellopsis prolifica* and 0.1M NaCl for *Scytonema cincinnatum* on the 42nd day of incubation (Table & Fig. 33).

Morphological studies revealed that the *Westiellopsis prolifica* does not lose its green colour even after 42nd day of incubation and it grows fastly on graded concentrations of NaCl with maximum number of heterocysts at 0.6M NaCl (Table & Fig. 34). Contrary to that *Scytonema cincinnatum* retards its growth from 21st day of incubation, it lost its colour and became yellowish at 0.7, 0.8 and 0.9 M NaCl. The filaments get disrupted and akinetes were produced on 14th day of incubation and the number of heterocysts was high at 0.6M NaCl and was maximum for the control. On 7th and 14th day of incubation the heterocyst number was high at 0.6M NaCl. On 21st day of incubation it was high at 0.4M and 0.5 M NaCl and on 42nd day of incubation, the heterocyst number was maximum at 0.1M and 0.2M NaCl respectively. From 0.6M to 0.9M NaCl, there were no increase in heterocyst number from the 7th day of incubation up to the end of experiment *i.e.*, 42nd day (Table & Fig. 35).

4.3.2. Discussion

Analysis of results showed that cyanobacteria of rice fields show a varying degree of tolerance to NaCl in culture which ranges from 100 to 900 mM. Even the organisms possessing a distinct sheath layer, in *Scytonema cincinnatum*, the cells were salt sensitive and showed that the external envelope layers did not play a significant role in osmoprotection. Earlier reports have shown that supplementation of 0.4M NaCl decreased biomass

accumulation, chlorophyll-a content and cell protein levels of *Calothrix brevissima* whereas *Anabaena* sp. of freshwaters tolerated only up to 0.1M NaCl (Kumar and Kaushik, 1994). The unicellular species *Synechocystis aquatilis* showed salt tolerance up to 0.3M NaCl whereas, *Microcystis firma* tolerated up to 1M NaCl. The growth of N_2 fixing cyanobacterium *Nostoc muscorum* was completely inhibited by 0.4M NaCl (Blumwald and Tel-Or, 1982a). But according to Joset *et al.* (1996) cyanobacteria can tolerate salinity levels from 10 mM to 3M salt in the environment. All these results showed the varying degree of tolerance to NaCl in different species.

Like the results of the present investigation on the changes of photosynthetic pigments, protein content, decrease in the number of heterocysts at higher salt concentrations is in accordance with the earlier findings that the presence of salt yields a series of processes affecting cell composition, structure and function such as stimulation of photosynthesis, accumulation of sugars, reorganisation of photosynthetic apparatus and modification of cell surface (Blumwald and Tel-Or, 1982a, b). The complete reorganization and redistribution of the thylakoids within the adapted cells involved reorganization of photosynthetic units and redistribution of pigments (Blumwald and Tel-Or, 1982a). The drastic reduction in chlorophyll content was correlated to higher Na^+ and Cl^- concentrations. Sodium is required for growth (Allen and Arnon, 1955; Kratz and Myers, 1955), nitrogenase activity (Apte and Thomas, 1980) and photosynthesis (Apte and Thomas, 1984). Sodium plays a multiple role in cyanobacteria and may be the main

causative factor for stimulated growth of *Westiellopsis prolifica* at low salt concentrations.

The present investigation on *Scytonema cincinnatum* agrees with the earlier findings that there will be severe disintegration of the cyanobacterium *Anabaena cylindrica* which was measured by filament breakage, cell disruption, phycocyanin release and nitrogenase inhibition by several commonly used buffers and NaCl (Smith *et al.*, 1983). There are similar reports in leaching of phycobilin pigments namely phycocyanin, phycoerythrin and allophycocyanin when the salinity was more than 250 mM NaCl in *Oscillatoria sancta* and in *Nostoc piscinale.* The failure of the organism to tolerate higher salinity was also related by a rapid drop in the photosynthetic oxygen evolution (Anand *et al.*, 1987). The present findings are in accordance with the earlier reports (Fay, 1969a) that the reduction in protein levels can be due to heavy pigment release as the phycobilin pigments constitute as much as 40% of the total soluble proteins in cyanobacteria.

There are several reports on several mechanisms put forth for salinity tolerance in cyanobacteria. These include the curtailment of Na^+ influx (Apte *et al.*, 1987), accumulation of k^+ (Miller *et al.*, 1984; Kaushik and Nager, 1993), synthesis and accumulation of osmoprotective compounds to achieve an equilibrium of osmotic potential (Reed *et al.*, 1986; Reed and Stewart, 1988). Salinity stress in cyanobacteria is also relieved by a variety of exogenous saccharides and alcohols. The low salinity induces soluble

sugars and inhibits starch content and thus extracellular saccharides and aminoacids secreted to the medium also provide protection to rice field cyanobacteria from NaCl stress (Reed *et al.*,1984; Padhi *et al.*, 1998). In addition, the tolerance is also achieved by maintaining a low ion concentration inside the cell by the use of active transport mechanisms like a Na^+/H^+ antiporter (Packer *et al.*, 1987), which is energized by the increased activity of the cytochrome oxidase (Molitor *et al.*, 1986) or H^+ ATPases (Gabbay-Azaria *et al.*, 1994). Further consequences of a salt shock observed in cyanobacteria are the induction of stress proteins (Bhagwat and Apte, 1989; Hagemann *et al.*, 1991), the short decrease of photosynthesis directly after the shock, the increase of cyclic electron flow around photosystem I (Jeanjean *et al.*, 1993), and changes in the composition of plasma membrane (Russell, 1989). Nomura *et al.* (1995) found that introduction of *bet*-genes encoding for enzymes involved in the synthesis of glycine betaine in *Escherichia coli* to the cyanobacterium *Synechococcus* sp. PCC 7942 and found that the synthesis of glycine betaine led to an increase in salt tolerance and better growth in a salt medium. The salt tolerance exhibited by many cyanobacteria has been exploited with some success in reclamation of saline and sodic soils (Kaushik and Venkataraman, 1982; Thomas and Apte, 1984). Application of cyanobacteria as biofertilizer to saline soil enhances the organic carbon content of soil due to the addition of enormous quantity of organic matter in the form of cyanobacterial biomass and reduce electrical conductivity of saline soil (Kannaiyan *et al.*, 1992c).

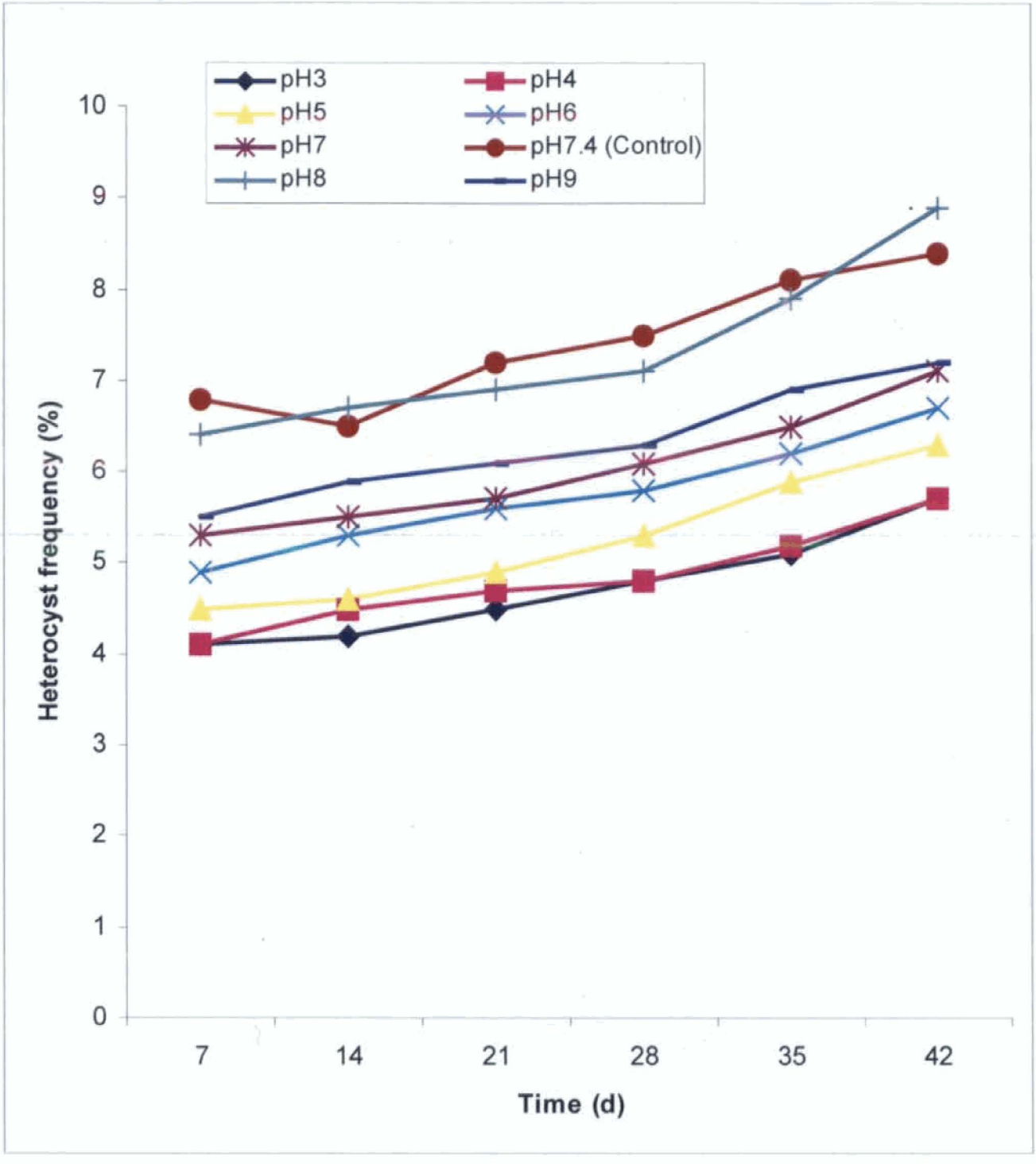


Fig.22 Effect of pH on heterocyst abundance of *Scytonema cincinnatum* up to 42 days of incubation.

Table 23. Effect of NaCl (0.1 M to 0.9 M) on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica*. Cultures were incubated for 42 days at 25 ± 1°C with 12 h light/12 h dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	Control
7	0.31 ± 0.02	0.31 ± 0.03	0.31 ± 0.02	0.32 ± 0.01	0.34 ± 0.03	0.36 ± 0.02	0.27 ± 0.04	0.25 ± 0.03	0.20 ± 0.01	0.30 ± 0.02
14	0.35 ± 0.06	0.36 ± 0.02	0.38 ± 0.02	0.41 ± 0.06	0.42 ± 0.04	0.47 ± 0.02	0.31 ± 0.03	0.30 ± 0.04	0.25 ± 0.02	0.38 ± 0.01
21	0.36 ± 0.04	0.38 ± 0.04	0.41 ± 0.06	0.43 ± 0.01	0.52 ± 0.02	0.58 ± 0.01	0.32 ± 0.02	0.31 ± 0.03	0.30 ± 0.02	0.46 ± 0.02
28	0.50 ± 0.04	0.54 ± 0.01	0.59 ± 0.01	0.62 ± 0.02	0.66 ± 0.02	0.71 ± 0.02	0.44 ± 0.02	0.43 ± 0.04	0.040 ± 0.02	0.52 ± 0.01
35	0.64 ± 0.05	0.73 ± 0.02	0.82 ± 0.02	0.91 ± 0.02	0.96 ± 0.01	1.08 ± 0.01	0.52 ± 0.02	0.49 ± 0.05	0.45 ± 0.03	0.63 ± 0.06
42	0.78 ± 0.06	0.80 ± 0.03	0.94 ± 0.03	0.95 ± 0.01	0.97 ± 0.01	1.11 ± 0.04	0.69 ± 0.01	0.64 ± 0.01	0.62 ± 0.03	0.74 ± 0.02

Values represent mean of three replicates ± S.D.

Table 24. Effect of NaCl (0.1 M to 0.9 M) on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum*. Cultures were incubated for 42 days at 25 ± 1°C with 12 h light/12 h dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	Control
7	0.31 ± 0.01	0.32 ±0.02	0.34 ± 0.03	0.35 ± 0.02	0.32 ± 0.02	0.30 ± 0.03	0.27 ± 0.03	0.031 ± 0.02	–	0.38 ± 0.02
14	0.33 ± 0.02	0.36 ± 0.02	0.37 ± 0.04	0.39 ± 0.01	0.34 ± 0.04	0.27 ± 0.01	0.35 ± 0.02	0.030 ± 0.01	–	0.40 ± 0.03
21	0.36 ± 0.03	0.38 ± 0.06	0.40 ± 0.03	0.46 ± 0.02	0.36 ± 0.05	0.29 ± 0.04	0.23 ± 0.06	0.01 ± 0.02	–	0.44 ± 0.04
28	0.40 ± 0.02	0.44 ± 0.04	0.45 ± 0.02	0.50 ± 0.03	0.23 ± 0.06	0.13 ± 0.02	0.009 ± 0.02	–	–	0.47 ± 0.02
35	0.43 ± 0.04	0.47 ± 0.02	0.52 ± 0.03	0.59 ± 0.02	0.14 ± 0.05	0.10 ± 0.01	–	–	–	0.55 ± 0.04
42	0.49 ± 0.04	0.54 ± 0.01	0.58 ± 0.06	0.64 ± 0.04	0.10 ± 0.07	0.006 ± 0.02	–	–	–	0.62 ± 0.05

Values represent mean of three replicates ± S.D.

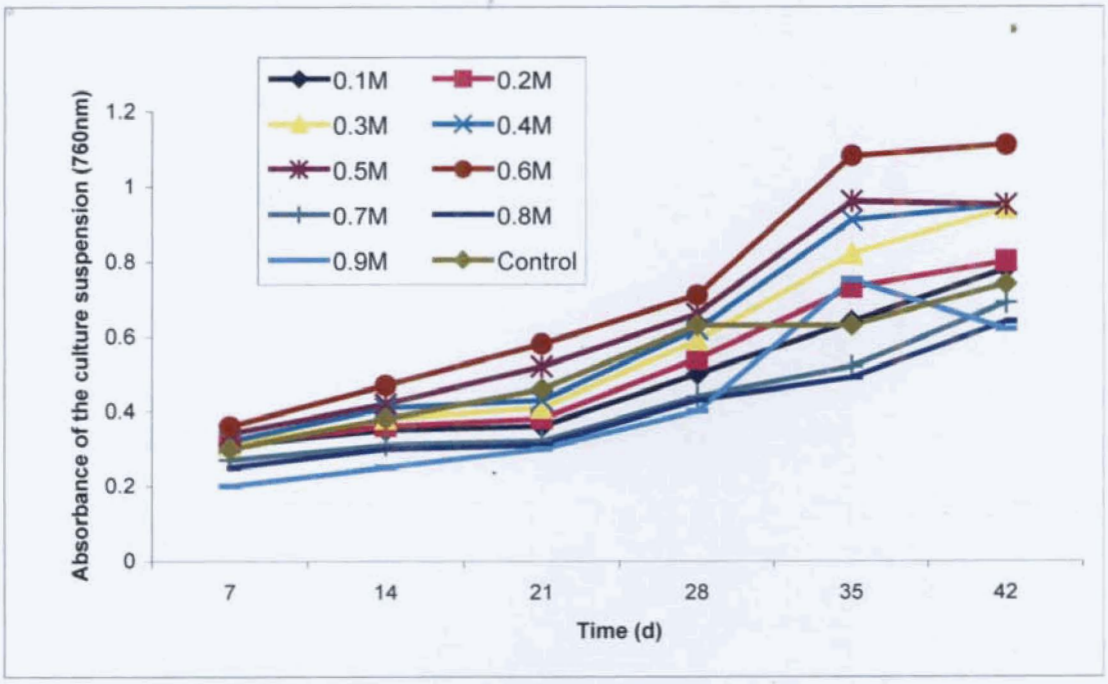


Fig.23 Effect of NaCl on the growth (absorbance of the culture suspension at 760nm) of *Westiellopsis prolifica* up to 42 days of incubation.

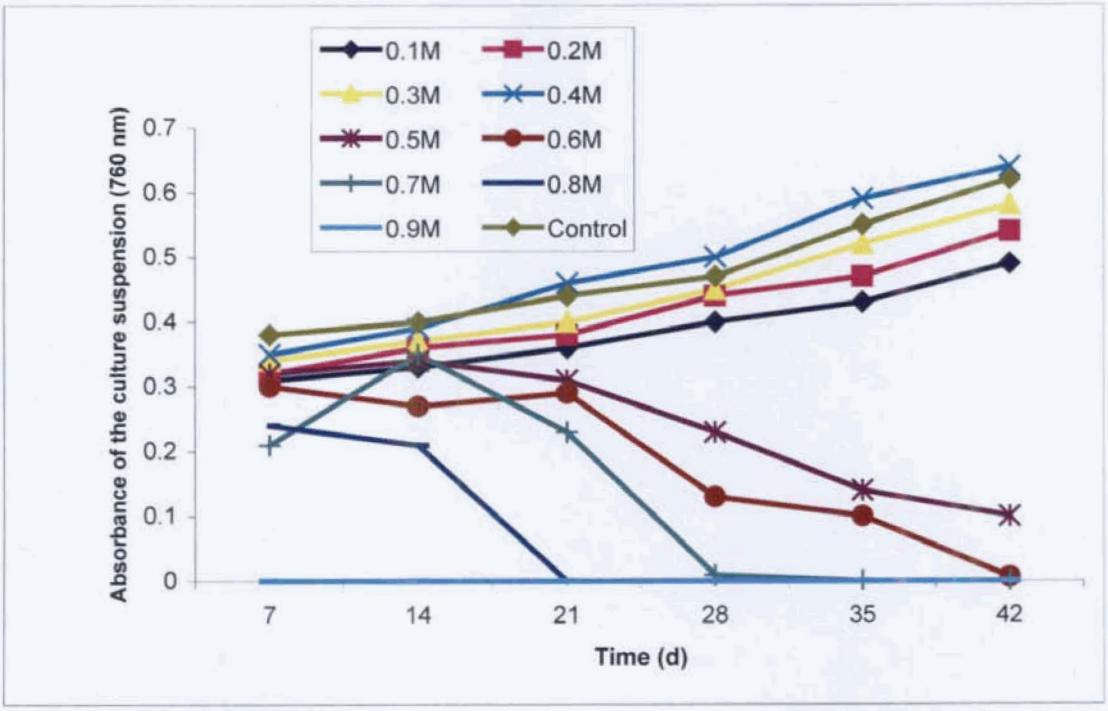


Fig.24 Effect of NaCl on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 42 days of incubation.

Table 25. Protein content (μg.ml^{-1} culture) of *Westiellopsis prolifica* at different concentrations of NaCl in the medium. Cultures were incubated at 25 ± 1°C up to 42 days with 12 h light/dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	21.21 ± 0.02	23.11 ± 0.01	25.16 ± 0.05	28.44 ± 0.1	36.31 ± 0.05	63.14 ±0.04	19.26 ± 0.02	15.12 ± 0.01	13.61 ± 0.02	58.12 ± 0.02
14	26.12 ± 0.05	28.34 ± 0.02	39.12 ± 0.08	46.32 ± 0.07	49.22 ± 0.01	56.32 ± 0.02	44.41 ± 0.04	32.22 ± 0.03	21.31 ± 0.01	62.46 ± 0.06
21	43.56 ± 0.07	42.83 ± 0.05	53.21 ± 0.06	65.23 ± 0.05	83.32 ± 0.05	94.1 ± 0.07	45.21 ± 0.03	36.48 ± 0.06	26.12 ± 0.05	71.28 ± 0.07
28	78.43 ± 0.03	83.16 ± 0.2	86.18 ± 0.01	98.48 ± 0.01	112.36 ±0.09	116.22 ± 0.02	82.12 ± 0.06	63.36 ± 0.04	62.36 ± 0.08	79.19 ± 0.03
35	82.16 ± 0.06	86.43 ± 0.01	93.26 ± 0.03	110.21 ± 0.01	114. 12± 0.08	141.31 ± 0.04	86.31 ± 0.02	68.18 ± 0.09	64.41 ± 0.07	83.13 ± 0.03
42	84.71 ± 0.02	92.32 ± 0.06	114.41 ± 0.08	132.36 ± 0.02	141.31 ± 0.01	160.36 ± 0.01	148.11± 0.03	96.24 ± 0.06	72.13 ± 0.02	90.47 ± 0.06

Values represent mean of three replicates ± S.D.

Table 26. Protein content (μg.ml^{-1} culture) of *Scytonema cincinnatum* at different concentrations of NaCl (0.1 to 0.9 M) in the medium. Cultures were incubated at 25 ± 1°C up to 42 days with 12 h light/dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	41.62 ± 0.02	47.23 ± 0.03	51.41 ± 0.02	55.16 ± 0.1	40.27 ± 0.01	38.62 ±0.02	36.17 ± 0.04	35.72 ± 0.02	28.37 ± 0.08	51.27 ± 0.02
14	42.36 ± 0.05	44.12 ± 0.02	47.32 ± 0.04	50.24 ± 0.01	38.36 ± 0.02	34.71 ± 0.02	32.36 ± 0.03	30.67 ± 0.02	21.46 ± 0.06	56.07 ± 0.03
21	38.31± 0.01	41.26 ± 0.03	44.1 ± 0.05	47.36 ± 0.06	36.35 ± 0.02	33.21 ± 0.05	33.72 ± 0.02	32.73 ± 0.04	18.14 ± 0.01	61.34 ± 0.03
28	35.62 ± 0.02	38.43 ± 0.02	40.2 ± 0.05	44.13 ± 0.05	31.21 ±0.03	25.62 ± 0.02	28.16 ± 0.02	28.27 ± 0.02	12.16 ± 0.01	72.11 ± 0.04
35	28.48 ± 0.02	34.26 ± 0.01	37.3 ± 0.01	41.27 ± 0.04	27.19 ± 0.03	21.71 ± 0.01	22.18 ± 0.05	15.12 ± 0.01	9.11 ± 0.05	76.45 ± 0.02
42	21.27 ± 0.01	25.36 ± 0.04	29.4 ± 0.01	37.36 ± 0.01	21.35 ± 0.02	19.26 ± 0.02	16.27 ± 0.01	12.17 ± 0.07	–	83.92 ± 0.04

Values represent mean of three replicates ± S.D.

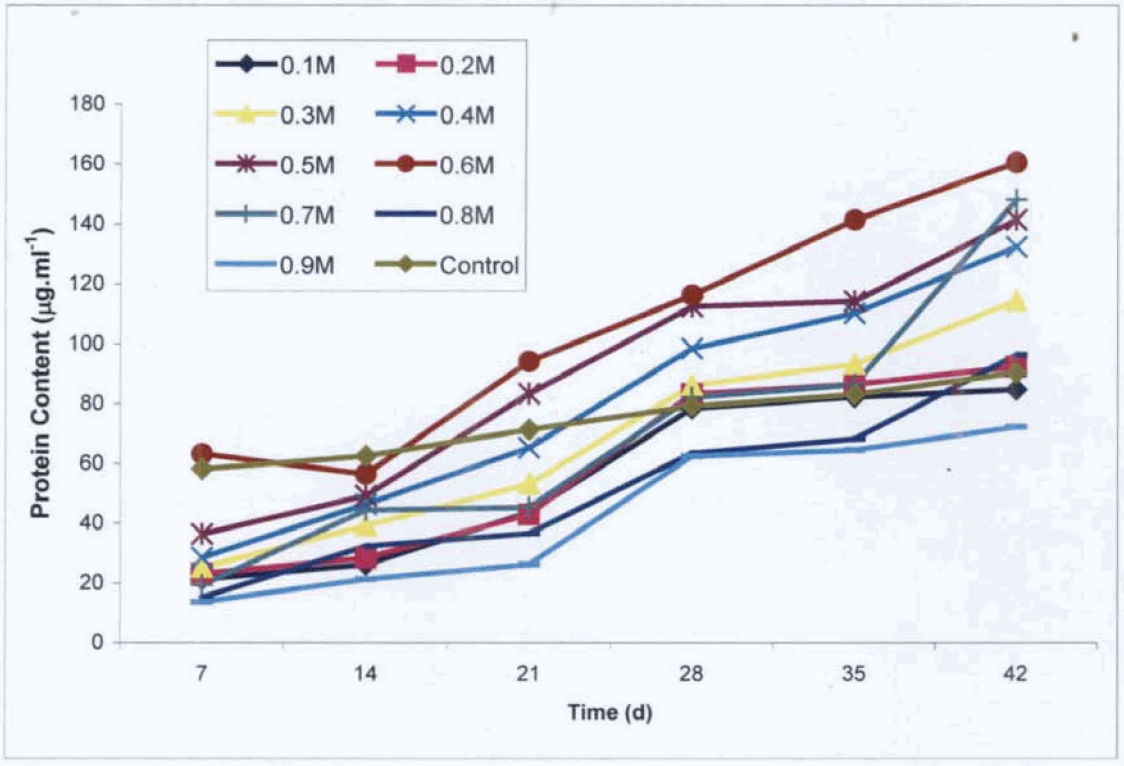


Fig.25 Effect of NaCl on protein content of *Westiellopsis prolifica* up to 42 days of incubation.

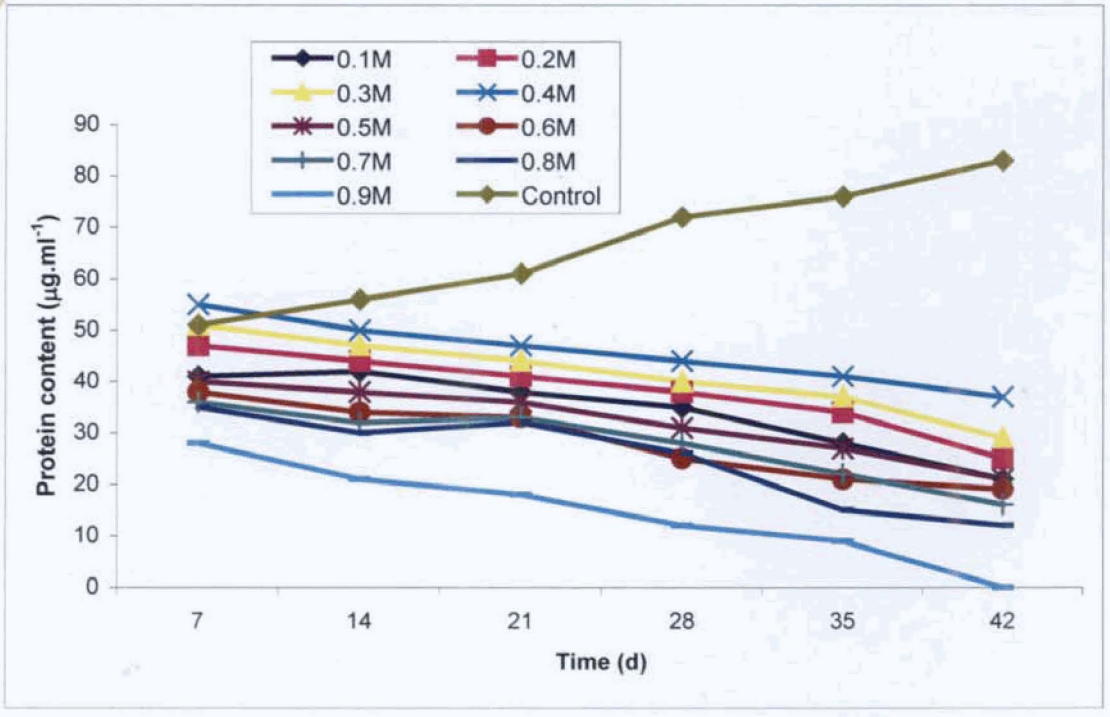


Fig.26 Effect of NaCl on protein content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 27. Effect of NaCl (0.1 M to 0.9M) on chlorophyll-a content (μg. ml^{-1} of culture) of *Westiellopsis prolifica* at 25 ± 1°C upto 42 days with 12 h light/dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	1.81 ± 0.07	1.83 ± 0.02	1.84 ± 0.06	2.45 ± 0.08	2.83 ± 0.06	2.94 ± 0.01	1.15 ± 0.01	1.04 ± 0.06	0.927 ± 0.07	1.72 ± 0.02
14	2.91 ± 0.01	2.95 ± 0.03	3.09 ± 0.02	3.46 ± 0.03	3.88 ± 0.05	4.12 ± 0.01	1.68 ± 0.06	1.19 ± 0.01	0.826 ± 0.01	3.01 ± 0.06
21	2.99 ± 0.02	3.04 ± 0.01	3.42 ± 0.03	3.81 ± 0.06	3.97 ± 0.05	4.41 ± 0.07	1.71 ± 0.07	1.62 ± 0.02	1.56 ± 0.08	3.23 ± 0.07
28	3.52 + 0.04	3.74 ± 0.03	3.97 ± 0.05	4.06 ± 0.02	4.61 ± 0.03	4.82 ± 0.06	2.46 ± 0.05	2.33 ± 0.06	2.01 ± 0.07	3.81 ± 0.01
35	4.12 ± 0.03	4.56 ± 0.02	4.94 ± 0.02	5.52 ± 0.03	6.54 ± 0.02	6.94 ± 0.02	3.01 ± 0.03	3.39 ± 0.02	3.19 ± 0.09	4.26 ± 0.09
42	4.67 ± 0.02	5.06 ± 0.06	6.21 ± 0.07	6.45 ± 0.06	6.83 ± 0.02	8.21 ± 0.03	4.52 ± 0.01	4.01 ± 0.02	3.83 ± 0.07	4.82 ± 0.08

Values represent mean of three replicates ± S.D.

Table 28. Effect of different concentrations of NaCl on the chlorophyll-a content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	1.044 ± 0.03	1.052 ± 0.05	1.058 ± 0.06	1.062 ± 0.01	1.079 ± 0.02	1.093 ± 0.07	1.088 ± 0.01	1.082 ± 0.1	1.071 ± 0.02	3.117 ± 0.09
14	2.126 ± 0.02	2.134 ± 0.02	2.137 ± 0.01	2.152 ± 0.16	2.157 ± 0.07	2.164 ± 0.06	1.121 ± 0.02	1.143 ± 0.02	1.102 ± 0.07	3.182 ± 0.01
21	2.135 ± 0.06	2.154 ± 0.04	2.187 ± 0.06	2.192 ± 0.07	1.126 ± 0.09	1.124 ± 0.07	1.101 ± 0.07	1.086 ± 0.06	1.072 ± 0.04	3.255 ± 0.07
28	2.196 ± 0.07	2.189 ± 0.08	2.201 ± 0.11	2.212 ± 0.14	1.133 ± 0.01	1.112 ± 0.06	1.045 ± 0.07	1.032 ± 0.06	1.027 ± 0.05	3.734 ± 0.02
35	2.232 ± 0.04	2.223 ± 0.06	2.201 ± 0.14	2.151 ± 0.21	1.107 ± 0.22	1.056 ± 0.01	--	--	--	3.921 ± 0.08
42	2.21 ± 0.05	2.193 ± 0.07	2.182 ± 0.02	2.112 ± 0.06	1.031 ± 0.01	1.023 ± 0.02	--	--	--	4.067 ± 0.06

Values represent mean of three replicates ± S.D.

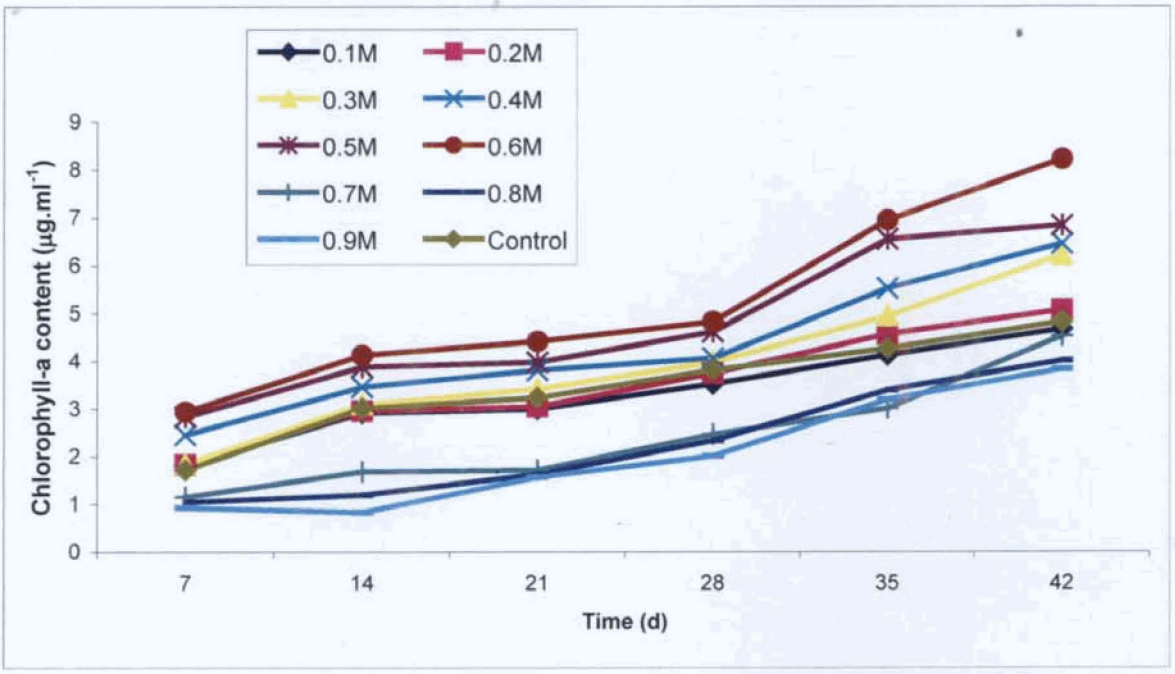


Fig.27 Effect of NaCl on chlorophyll-a content of *Westiellopsis prolifica* upto 42 days of incubation.

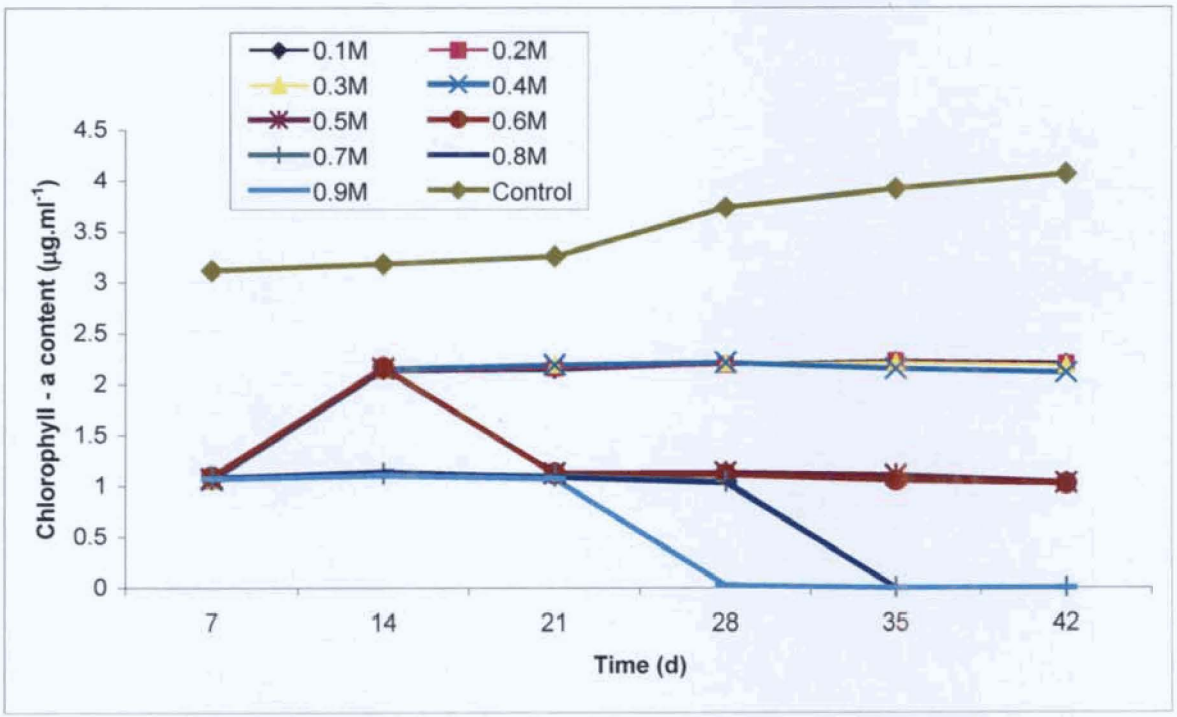


Fig 28 Effect of NaCl on chlorophyll-a content of *Scytonema cincinnatum* up to 42 days of incubation.

Table: 29. Effect of NaCl (0.1 to 0.9 M) on carotene content (μg.ml^{-1} culture) of *Westiellopsis prolifica*. Cultures were incubated for 42 days at 25 ± 1°C with 12 h light/dark cycle

Concentration of NaCl (M)										
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	0.587 ± 0.06	0.598 ± 0.04	0.623 ± 0.03	0.654 ± 0.05	0.683 ± 0.03	0.724 ± 0.04	0.711 ± 0.02	0.702 ± 0.05	0.699 ± 0.03	0.683 ± 0.04
14	0.592 ± 0.04	0.613 ± 0.03	0.638 ± 0.05	0.667 ± 0.04	0.694 ± 0.02	0.731 ± 0.04	0.715 ± 0.04	0.710 ± 0.03	0.707 ± 0.03	0.724 ± 0.05
21	0.598 ± 0.02	0.620 ± 0.01	0.644 ± 0.05	0.692 ± 0.03	0.724 ± 0.03	0.749 ± 0.02	0.720 ± 0.06	0.721 ± 0.02	0.713 ± 0.04	0.746 ± 0.04
28	0.610 ± 0.05	0.625 ± 0.04	0.649 ± 0.03	0.698 ± 0.04	0.729 ± 0.05	0.771 ± 0.03	0.727 ± 0.05	0.719 ± 0.02	0.716 ± 0.02	0.751 ± 0.05
35	0.615 ± 0.04	0.629 ± 0.06	0.667 ± 0.02	0.710 ± 0.05	0.735 ± 0.03	0789 ± 0.05	0.729 ± 0.01	0.721 ± 0.04	0.616 ± 0.06	0.762 ± 0.06
42	0.618 ± 0.03	0.638 ± 0.02	0.674 ± 0.04	0.719 ± 0.06	0.739 ± 0.02	0.799 ± 0.06	0.730 ± 0.05	0.723 ± 0.06	0.717 ± 0.02	0.775 ± 0.03

Values represent mean of three replicates ± S.D.

Table 30. Effect of NaCl (0.1 to 0.9 M) on carotene content (μg.ml^{-1} culture) of *Scytonema cincinnatum*. Cultures were incubated for 42 days at 25 ± 1°C with 12 h light / 12 h dark cycle

Concentration of NaCl (M)										
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	0.205 ± 0.03	0.210 ± 0.02	0.215 ± 0.04	0.222 ± 0.02	0.229 ± 0.04	0.242 ± 0.02	0.236 ± 0.03	0.231 ± 0.04	0.201 ± 0.03	0.296 ± 0.04
14	0.213 ± 0.03	0.216 ± 0.03	0.218 ± 0.02	0.224 ± 0.05	0.221 ± 0.06	0.206 ± 0.03	0.195 ± 0.04	0.165 ± 0.03	0.161 ± 0.02	0.314 ± 0.05
21	0.225 ± 0.05	0.229 ± 0.06	0.220 ± 0.03	0.228 ± 0.04	0.224 ± 0.05	0.199 ± 0.06	0.178 ± 0.06	0.142 ± 0.05	0.133 ± 0.05	0.352 ± 0.06
28	0.229 ± 0.02	0.231 ± 0.04	0.234 ± 0.02	0.231 ± 0.06	0.216 ± 0.04	0.172 ± 0.04	0.161 ± 0.07	0.135 ± 0.04	0.131 ± 0.04	0.395 ± 0.04
35	0.240 ± 0.03	0.240 ± 0.02	0.251 ± 0.03	0.242 ± 0.04	0.217 ± 0.04	0.163 ± 0.05	0.122 ± 0.08	0.105 ± 0.06	0.092 ± 0.03	0.465 ± 0.03
42	0.243 ± 0.04	0.241 ± 0.03	0.240 ± 0.05	0.236 ± 0.03	0.219 ± 0.03	0.134 ± 0.02	0.081 ± 0.02	0.074 ± 0.02	0.056 ± 0.02	0.521 ± 0.03

Values represent mean of three replicates ± S.D.

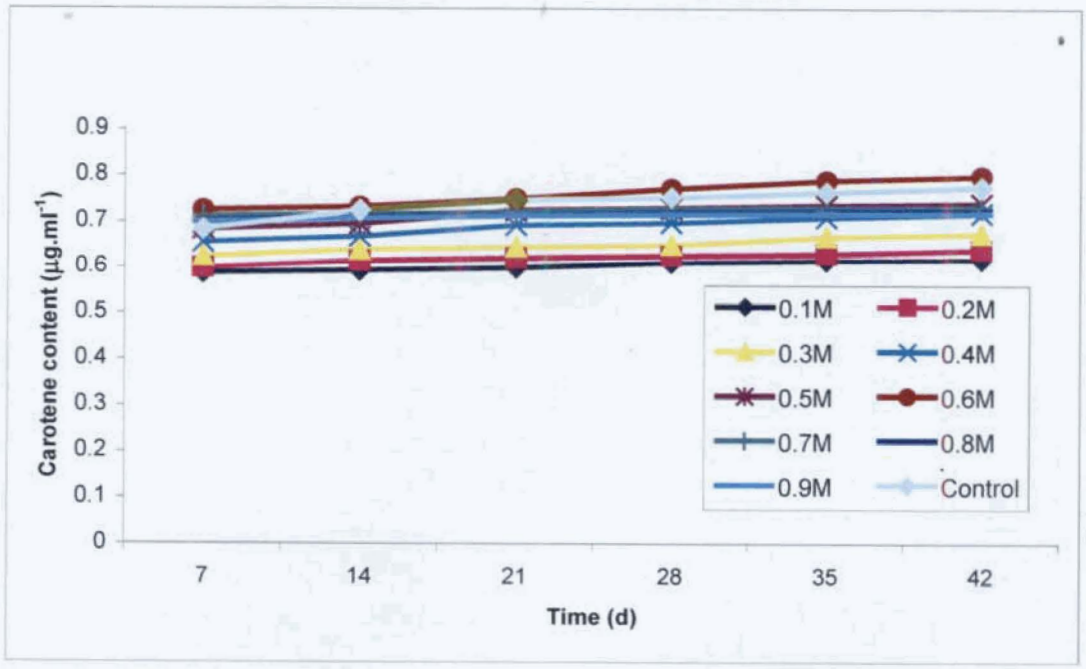


Fig.29 Effect of NaCl on carotene content of *Westiellopsis prolifica* up to 42 days of incubation.

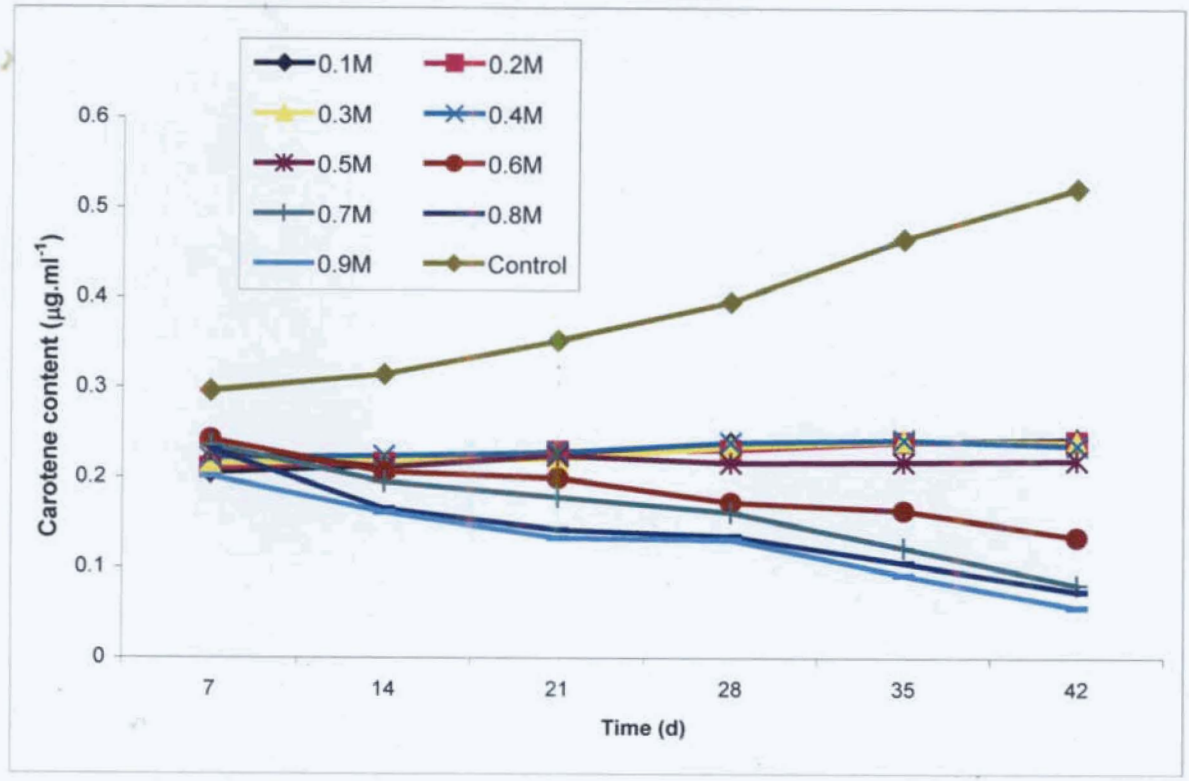


Fig.30 Effect of NaCl on carotene content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 31. Phycobiliprotein content (μg.ml^{-1} culture) of *Westiellopsis prolifica* at different concentrations of NaCl in the medium on 42nd day of incubation

	Concentration of NaCl (M)									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
APC	25.72 ± 0.18	25.83 ± 0.06	25.92 ± 0.09	25.98 ± 0.15	26.01 ± 0.27	27.36 ± 0.13	27.94 ± 0.35	28.14 ± 0.37	24.31 ± 0.19	25.32 ± 0.15
PC	31.62 ± 0.23	32.08 ± 0.21	32.17 ± 0.16	32.33 ± 0.13	32.54 ± 0.32	32.86 ± 0.26	33.12 ± 0.12	33.48 ± 0.31	34.51 ± 0.18	34.11 ± 0.09
PE	1.18 ± 0.21	1.21 ± 0.19	1.25 ± 0.11	1.31 ± 0.16	1.42 ± 0.23	1.53 ± 0.09	1.74 ± 0.22	1.96 ± 0.07	2.01 ± 0.16	2.12 ± 0.13

Values represent mean of three replicates ± S.D.

Table 32. Phycobiliprotein content (μg.ml^{-1} culture) of *Scytonema cincinnatum* at different concentrations of NaCl in the medium on the 42nd day of incubation

	Concentration of NaCl (M)									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
PC	22.11 ± 0.05	24.42 ± 0.09	25.59 ± 0.17	26.04 ± 0.07	30.32 ± 0.26	32.68 ± 0.08	33.54 ± 0.07	36.01 ± 0.19	39.46 ± 0.21	23.01 ± 0.06
APC	20.36 ± 0.09	22.42 ± 0.11	24.98 ± 0.06	25.35 ± 0.09	29.86 ± 0.08	28.11 ± 0.11	30.34 ± 0.18	34.56 ± 0.13	37.99 ± 0.27	20.32 ± 0.14
PE	1.598 ± 0.07	1.598 ± 0.09	1.609 ± 0.12	1.612 ± 0.14	2.614 ± 0.19	2.617 ± 0.06	2.620 ± 0.03	2.623 ± 0.02	2.627 ± 0.05	1.644 ± 0.08

Values represent mean of three replicates ± S.D.

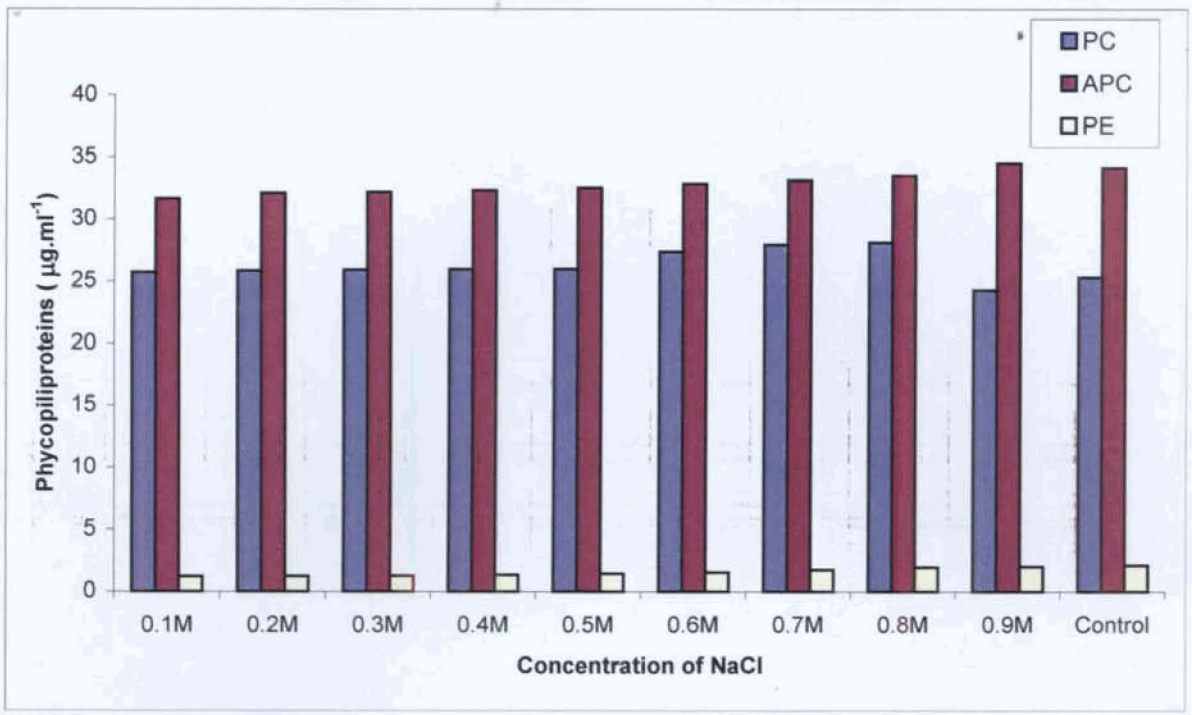


Fig.31 Effect of NaCl on phycobiliprotein content of *Westiellopsis prolifica* on 42nd day of incubation.

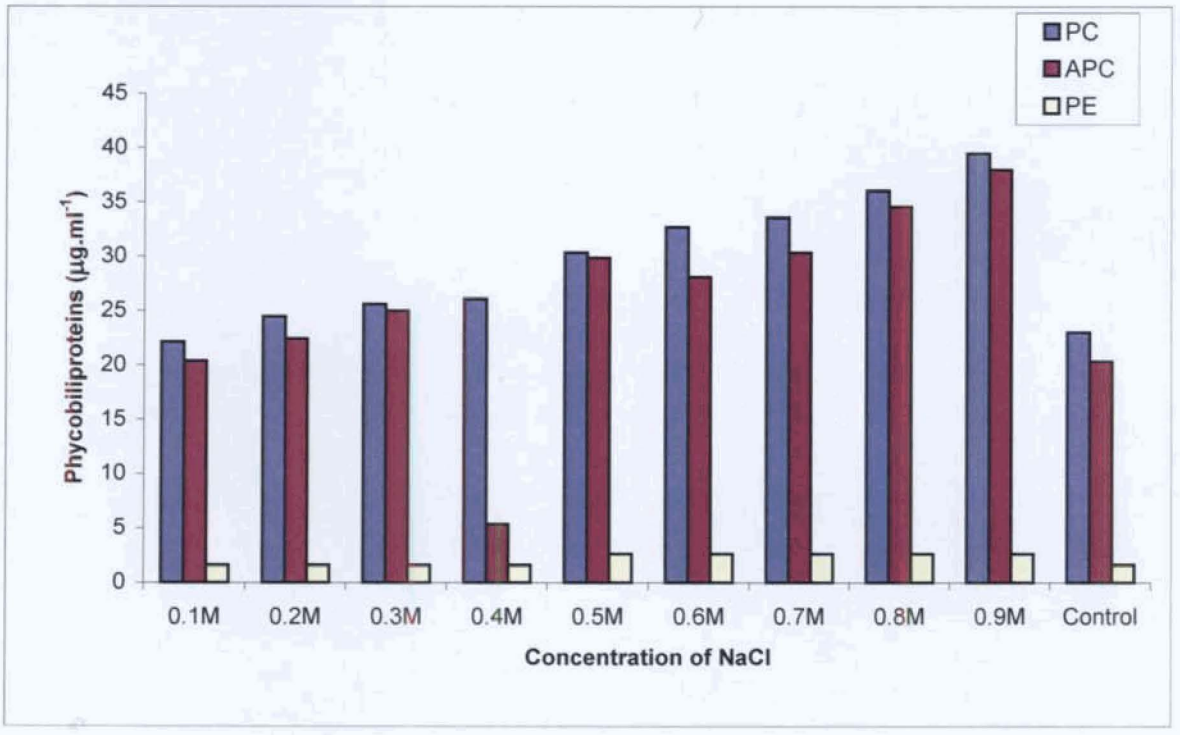


Fig.32 Effect of NaCl on phycobiliprotein content of *Scytonema cincinnatum* on 42nd day of incubation.

Table 33. Effect of NaCl (0.1 M to 0.9 M) on ammonia excretion (n moles ml^{-1}) of culture of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation

	Concentration of NaCl (M)									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
Westiellopsis prolifica	21.5 ± 1.2	21.7 ± 1.3	22.4 ± 0.05	22.6 ± 2.1	23.3 ± 1.5	24.0 ± 2.2	15.5 ± 2.6	14.1 ± 1.7	13.5 ± 1.8	21.1 ± 1.4
Scytonema cincinnatum	14.2 ± 0.81	13.3 ± 2.3	12.5 ± 1.7	17.7 ± 0.91	10.3 ± 2.6	7.8 ± 2.1	7.1 ± 0.05	6.2 ± 0.19	5.3 ± 0.27	16.7 ± 0.36

Values represent mean of three replicates ± S.D.

Table 34: Heterocyst frequency (%) of *Westiellopsis prolifica* at different concentrations of NaCl (0.1 to 0.9M) in the medium. Cultures were incubated at 25 ± 1°C up to 42 days with 12 h light/dark cycle

	Concentration of NaCl (M)									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	5.8 ± 0.76	6.3 ± 0.23	6.8 ± 0.12	7.4 ± 0.33	7.9 ± 0.18	8.6 ± 0.27	45 ± 0.39	4.1 ± 0.28	3.2 ± 0.37	7.1 ± 0.09
14	6.4 ± 0.18	6.6 ± 0.11	6.9 ± 0.32	7.6 ± 0.87	8.2 ± 0.35	10.3 ± 0.21	5.2 ± 0.38	4.3 ± 0.48	3.3 ± 0.51	8.3 ± 0.17
21	7.2 ± 0.42	7.3 ± 0.19	8.4 ± 0.06	8.6 ± 0.12	8.7 ± 0.12	11.7 ± 0.15	6.4 ± 0.05	5.1 ± 0.46	3.9 ± 0.07	11.1 ± 0.06
28	10.8 ± 0.06	11.6 ± 0.26	12.1 ± 0.21	12.3 ± 0.18	12.6 ± 0.14	12.9 ± 0.25	6.9 ± 0.07	5.3 ± 0.16	4.1 ± 0.11	12.2 ± 0.16
35	11.1 ± 0.11	11.9 ± 0.09	12.1 ± 0.17	12.5 ± 0.21	12.9 ± 0.09	13.8 ± 0.31	7.2 ± 0.07	5.3 ± 0.13	4.2 ± 0.08	12.4 ± 0.19
42	11.6 ± 0.03	12.1 ± 0.06	12.4 ± 0.15	12.8 ± 0.07	13.1 ± 0.06	14.1 ± 09	7.1 ± 0.11	5.4 ± 0.06	4.2 ± 0.03	11.6 ± 0.13

Values represent mean of three replicates ± S.D.

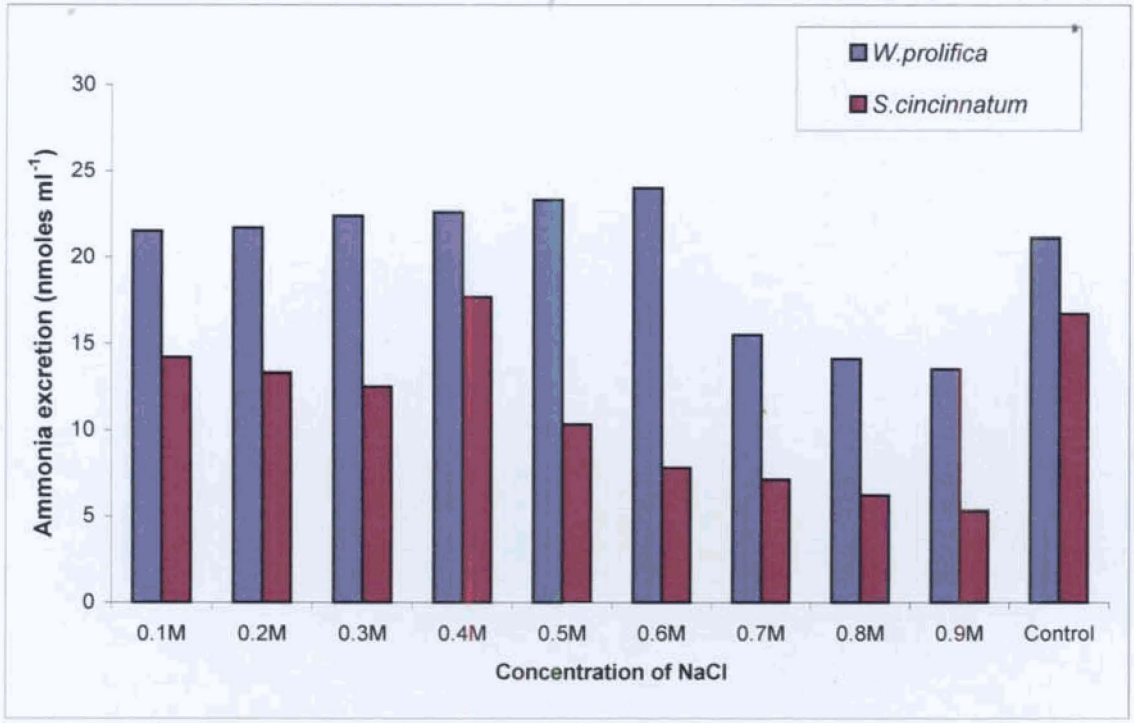


Fig.33 Effect of NaCl on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation.

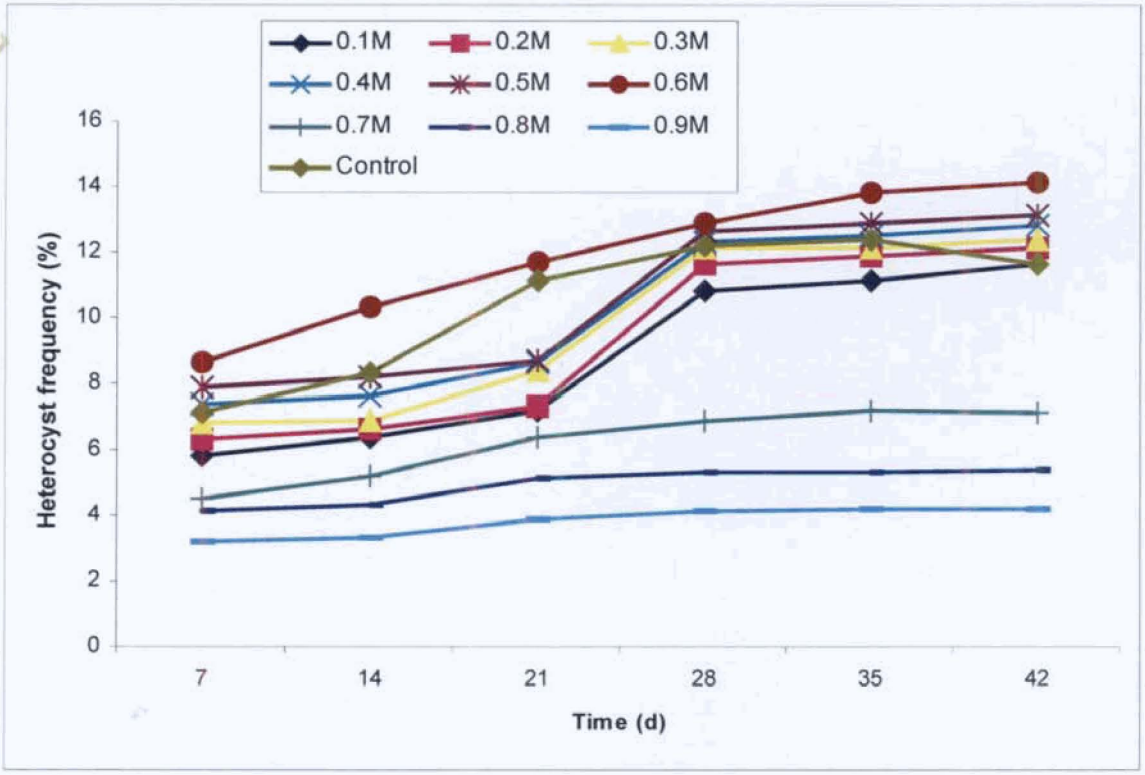


Fig.34 Heterocyst frequency (%) of *Westiellopsis prolifica* at various concentration of NaCl upto 42 days of incubation.

Table 35: Heterocyst frequency (%) of *Scytonema cincinnatum* at different concentrations of NaCl (0.1 to 0.9M) in the medium. Cultures were incubated at 25 ± 1°C up to 42 days with 12 h light/dark cycle

	Concentration of NaCl (M)									
No. of days	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	C
7	2.5 ± 0.12	2.7 ± 0.13	3.2 ± 0.07	3.3 ± 0.27	3.5 ± 0.08	3.8 ± 0.12	3.7 ± 0.19	3.6 ± 0.17	3.1 ± 0.11	6.2 ± 0.08
14	2.9 ± 0.27	3.1 ± 0.23	3.4 ± 0.16	3.5 ± 0.08	3.9 ± 0.02	4.2 ± 0.07	3.1 ± 0.17	2.9 ± 0.12	2.5 ± 0.06	6.5 ± 0.17
21	3.3 ± 0.18	3.5 ± 0.27	3.8 ± 0.09	4.2 ± 0.01	4.1 ± 0.09	3.9 ± 0.12	3.2 ± 0.21	2.1 ± 0.28	1.9 ± 0.22	7.3 ± 0.19
28	3.6 ± 0.09	3.7 ± 0.05	4.1 ± 0.01	4.2 ± 0.05	4.1 ± 0.23	2.3 ± 0.33	2.1 ± 0.17	1.8 ± 0.11	1.3 ± 0.16	7.9 ± 0.03
35	3.8 ± 0.17	3.8 ± 0.08	3.8 ± 0.02	3.9 ± 0.09	3.8 ± 0.07	1.8 ± 0.27	1.5 ± 0.23	1.3 ± 0.21	1.2 ± 0.19	8.6 ± 0.08
42	4.1 ± 0.23	4.1 ± 0.06	3.9 ± 0.21	3.8 ± 0.06	3.5 ± 0.08	1.8 ± 0.03	1.4 ± 0.28	1.2 ± 0.21	1.2 ± 0.06	9.1 ± 0.03

Values represent mean of three replicates ± S.D.

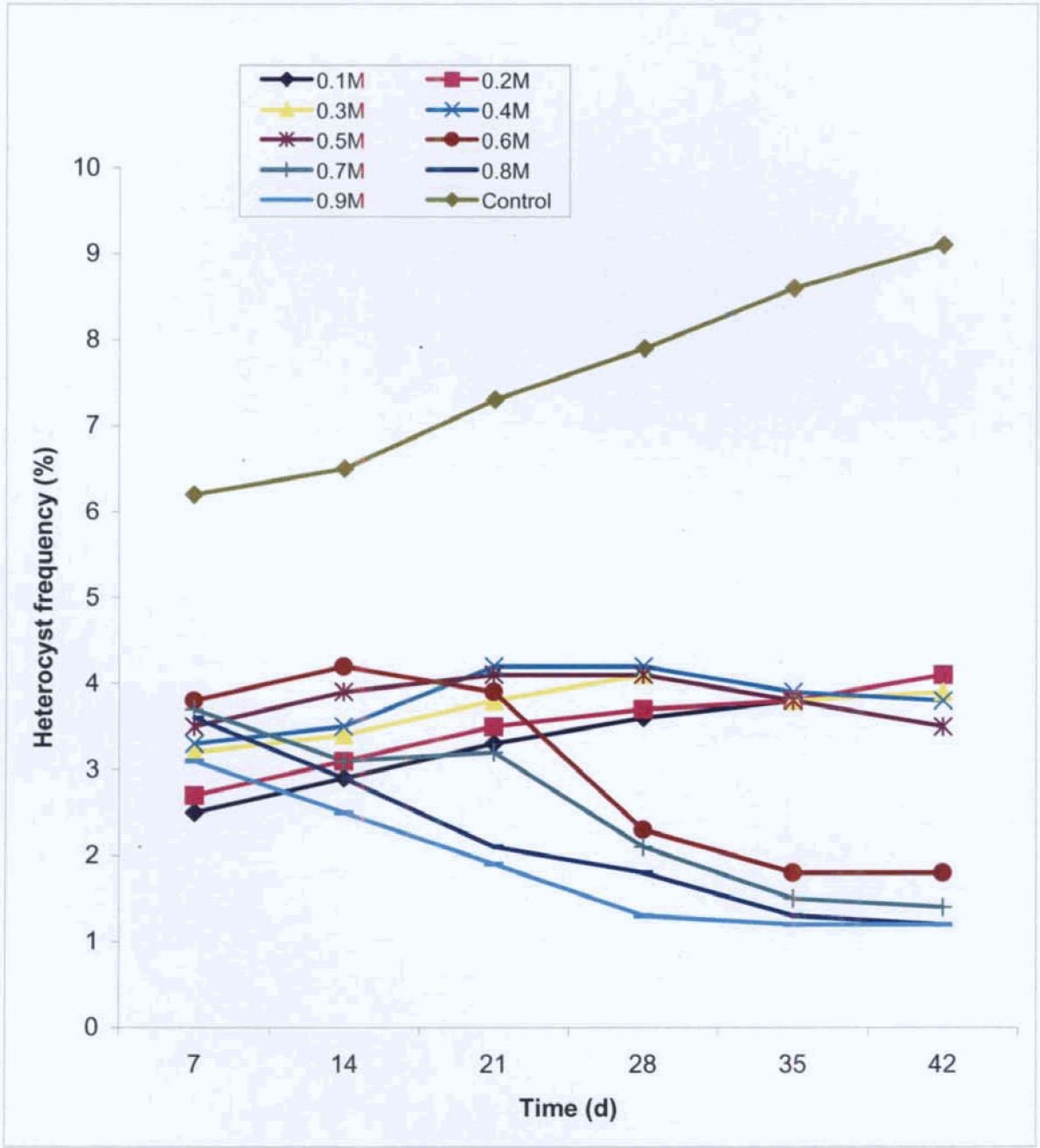


Fig.35 Heterocyst frequency of *Scytonema cincinnatum* at various concentrations of NaCl up to 42 days of incubation.

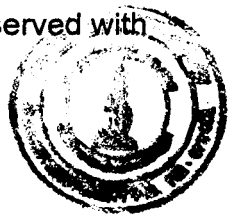
Recently, Apte and Thomas (1997) found that more than 90% of the cell bound sodium remained extracellularly trapped in the slime layers of cyanobacteria and the top soils containing cyanobacterial mats decreased the salinity of the soil up to 38 per cent. Thus it was clear that the application of saline tolerant cyanobacteria for amelioration of soil salinity is an attractive possibility and can be used as biofertilizer in those areas where the rice fields are subjected to fluctuating salinity.

4.4. RESPONSE OF CYANOBACTERIA TO EXOGENOUS CARBON COMPOUNDS – MIXOTROPHIC, PHOTOHETEROTROPHIC AND CHEMOHETEROTROPHIC GROWTH BY *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM*

4.4.1. Results

Growth as well as ammonia excretion of *Westiellopsis prolifica* and *Scytonema cincinnatum* under photoheterotrophic and chemoheterotrophic growth conditions in presence of two different sugars, *viz.*, glucose and sucrose (15mM each) was studied. The capability to utilize a specific sugar under definite growth conditions varied between two genera (Table 36, 37 & Fig. 36-39). *Scytonema cincinnatum* prefers mixotrophic growth in which growth was higher in exogenous carbon source in light than control while chemoheterotrophic growth was completely absent. In *Westiellopsis prolifica* both mixotrophic and chemoheterotrophic growth was noticed in which sucrose was the most prefered substrate than glucose. Though microscopic examination of the cultures revealed significant variations in the branching

frequencies, no unhealthy filaments of the cyanobacteria were observed with different treatments.



4.4.2. Discussion

Primary mode of growth in cyanobacteria is photoautotrophy and are unable to grow heterotrophically in darkness (Hoare *et al.*, 1967; Holm – Hansen, 1968). But there are several reports that cyanobacteria can grow heterotrophically at the expense of exogenous carbon sources (Smith, 1973; Khoja and Whitton, 1975; Adhikary and Pattnaik, 1979; Rippka *et al.*, 1979; Ramos *et al.*, 1985; Banerjee and Kumar, 1987; Bastia *et al.*, 1993). However, those strains of cyanobacteria so far demonstrated to be capable of growing heterotrophically can do so only about a fraction of their photoautotrophic growth rates. In the present study, the cyanobacterial species grew well and synthesized photosynthetic pigment chlorophyll-a at a higher rate in presence of 15 mM glucose and sucrose in the culture medium in light in comparison to their respective control cultures.

Though cyanobacteria can utilize a wide spectrum of exogenous carbon compounds, both in light and darkness, there are only few substrates, mostly monosaccharides, which can act as substrates for growth in darkness, in addition to their normal autotrophic mode of growth in light (Smith, 1973, 1982; Barnum and Gendel, 1987). The sugar utilization rate in *Westiellopsis prolifica* and *Scytonema cincinnatum* under mixotrophic conditions results in an increase in growth rate and was higher than the autotrophic and chemoheterotrophic growth. But the substrate specificity

differs in different genera. In *Westiellopsis prolifica* growth was more in sucrose substrate while in *Scytonema cincinnatum* glucose favours increased growth. This suggests that the substrate specificity and efficiency of substrate utilization are manifestations of growth conditions. It is conceivable that light and dark treatments may bring alternations in the membrane permeability and transport properties for assimilation of organic substrate. Thus a specific substrate supported better growth of an organism in light may not possess the same ability to support its photoautotrophic and chemoautotrophic growth. The findings of the present investigation suggest that glucose and sucrose were efficient in allowing the growth of both cyanobacteria in light or in dark are in accordance with the earlier findings on *Tolypothrix tenuis* (Kiyohara *et al.*, 1960), *Anabaenopsis circularis* (Watanabe and Yamamoto, 1967), *Plectonema boryanum* (White and Shilo, 1975) and various other cyanobacteria (Bastia *et al.*, 1993). Since *Scytonema cincinnatum* could utilize either of the substrate for their growth in light and *Westiellopsis prolifica* in both light and darkness, the heterotrophic growth rate was never comparable to their respective autotrophic growth rates suggesting that it can capable of growing facultative heterotrophically utilizing specific exogenous carbon compounds.

Table 36. Effect of exogenous sugars (15 mM) on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum*. Cultures were incubated for 15 days at 25 ± 1°C with 12 h light/dark cycle or in dark

	Light			Dark		
	Control	Glucose	Sucrose	Control	Glucose	Sucrose
W. prolifica	0.34 ± 0.09	0.53 ± 0.05	0.61 ± 0.07	0.20 ± 0.05	0.23 ± 0.06	0.31 ± 0.05
S. cincinnatum	0.29 ± 0.06	0.56± 0.07	0.44 ± 0.05	0.20 ± 0.04	0	0

Values represent mean of three replicates ± S.D.

Table 37. Effect of exogenous sugars (15 mM) on chlorophyll-a content (μg. ml^{-1} culture) of *Westiellopsis prolifica* and *Scytonema cincinnatum*. Cultures were incubated for 15 days at 25 ± 1°C with 12 h light/dark cycle or in dark

	Light			Dark		
	Control	Glucose	Sucrose	Control	Glucose	Sucrose
W. prolifica	2.75 ± 0.11	3.76 ± 0.09	4.02 ± 0.08	0.11 ± 0.07	0.16 ± 0.08	0.22 ± 0.06
S. cincinnatum	2.08 ± 0.05	3.95 ± 0.07	3.27 ± 0.05	0	0	0

Values represent mean of three replicates ± S.D.

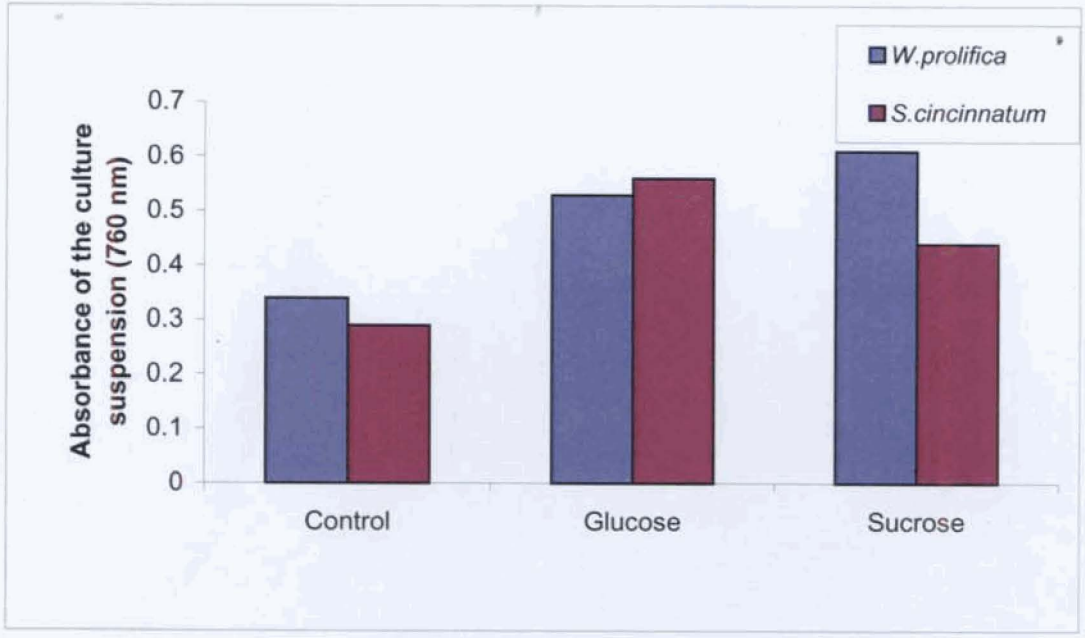


Fig. 36 Effect of exogenous carbon compounds on growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 15th day of incubation in light

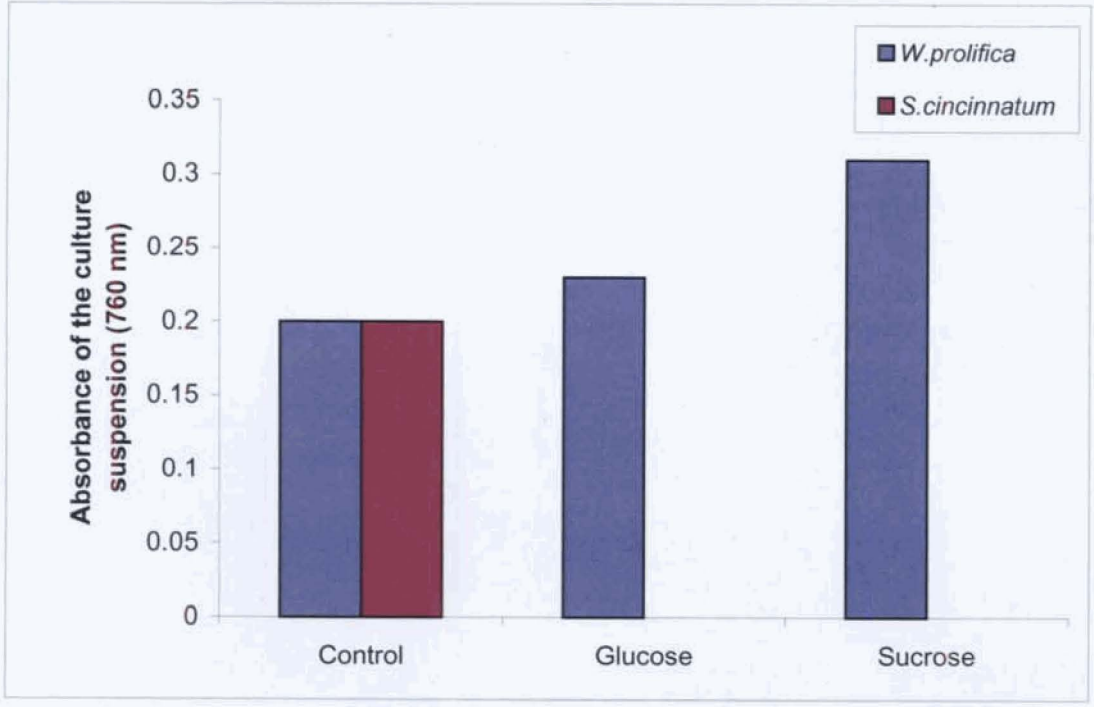


Fig.37 Effect of exogenous carbon compounds on growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 15th day of incubation in dark.

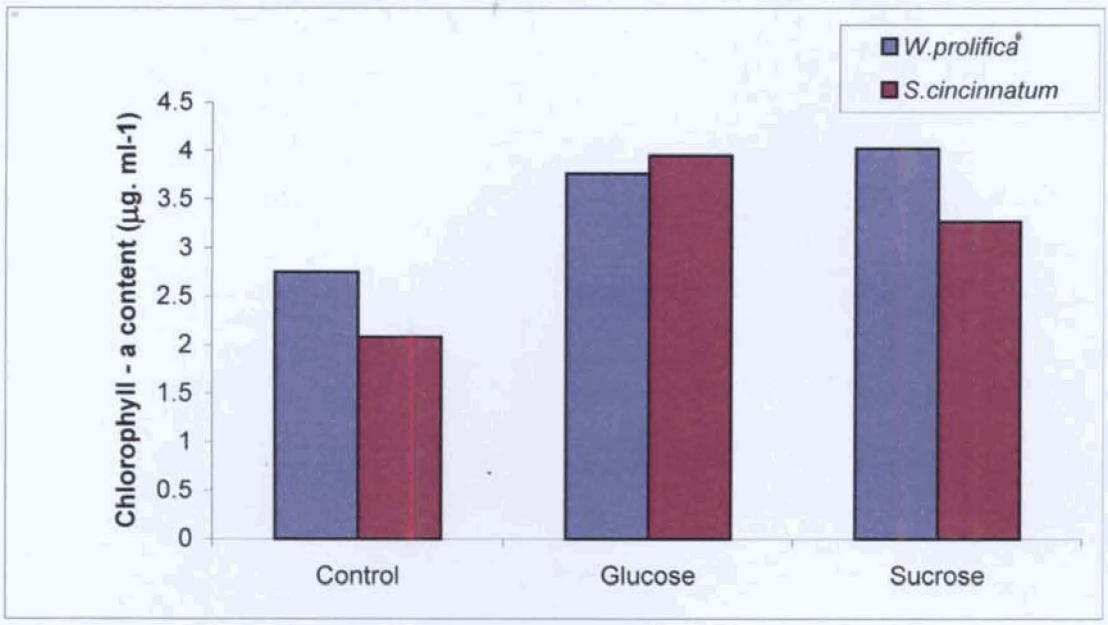


Fig. 38 Effect of exogenous carbon compounds on chlorophyll-a content of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 15th day of incubation in light

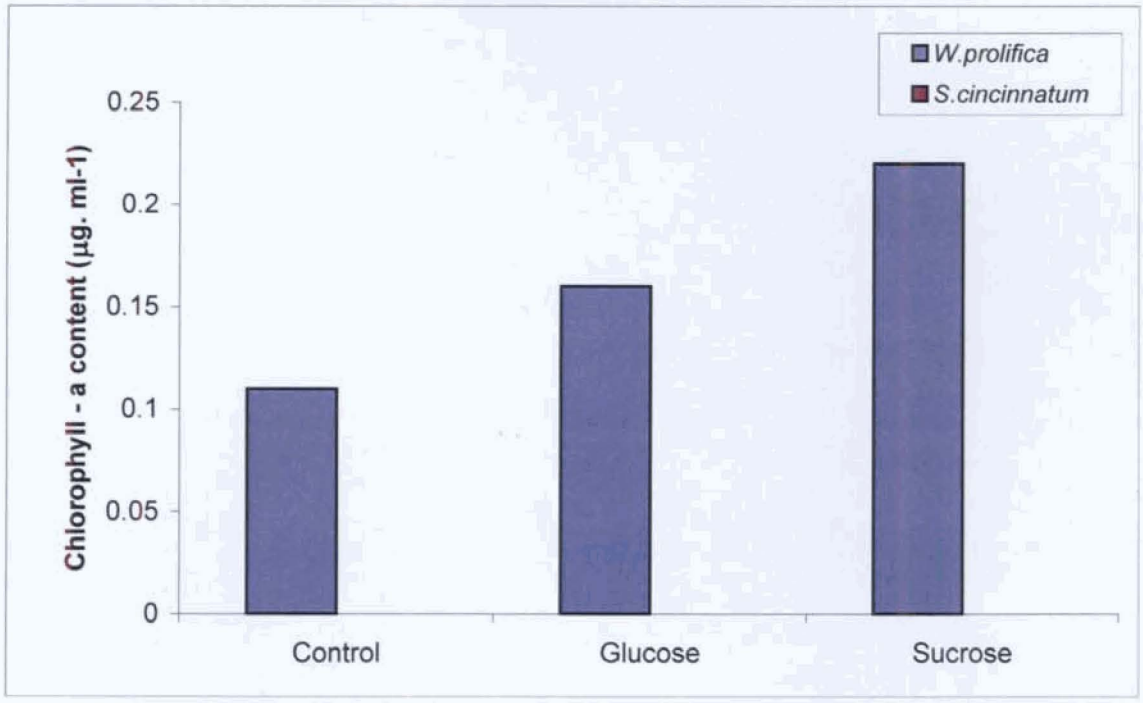


Fig.39 Effect of exogenous carbon compounds on chlorophyll-a content of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 15th day of incubation in dark.

4.5. EFFECT OF CARBOFURAN ON THE GROWTH AND BIOCHEMICAL CHARACTERS OF *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM*

4.5.1. Results

The growth and biochemical characters of *Westiellopsis prolifica* and *Scytonema cincinnatum* are presented in the Tables 38-49. The cyanobacterial strains recorded a considerable growth in different concentrations of carbofuran. In *Westiellopsis prolifica* and in *Scytonema cincinnatum*, the growth was maximum at 50 ppm and 30 ppm respectively (Table 38, 39 & Fig. 40, 41). In *Westiellopsis prolifica* , the chlorophyll-a content was significantly high at carbofuran treated cultures than the control and maximum at 50 ppm eventhough it can thrive up to 100 ppm. There was a sudden increase in chlorophyll-a content after two weeks of inoculation up to 70 ppm over the control. At 80 ppm and 90 ppm the chlorophyll-a content decreased considerably. At 100 ppm, very minute increase in chlorophyll-a was noticed (Table 40 & Fig. 42).

In *Scytonema cincinnatum*, the maximum chlorophyll-a content was noticed at 30 ppm eventhough it can thrive up to 100 ppm. At 100 ppm there was no significant increase in chlorophyll-a content (Table 41 & Fig. 43). The carotene content increased up to 70 ppm over the control and was maximum at 50 ppm in *Westiellopsis prolifica.* But at 100 ppm there was an increase in carotene content up to 42 days of incubation (Table 42 & Fig. 44). In *Scytonema cincinnatum* carotene content was maximum at 30 ppm

carbofuran (Table 43 & Fig. 45). Total protein content of *Westiellopsis prolifica* was significantly higher in carbofuran treated cultures at 50 ppm and at 100 ppm it reduces to half over the control (Table 44 & Fig. 46). While in *Scytonema cincinnatum*, maximum protein content was noticed at 30 ppm over the control. At 40 ppm to 100 ppm very minute increase in protein content was observed upto 42 days of incubation (Table 45 & Fig. 47). The phycobilins namely c-phycocyanin, allophycocyanin and c-phycoerythrin levels were slightly higher than control and showed similar pattern of increase with maximum at 50 ppm and 30 ppm for *Westiellopsis prolifica* and *Scytonema cincinnatum* respectively. At higher concentrations of carbofuran, the phycobilin content decreased considerably and levelled off (Table 46, 47 & Fig. 48, 49). The ammonia production was significantly high at carbofuran treated cultures than control and was maximum at 50 ppm and 30 ppm for *Westiellopsis prolifica* and *Scytonema cincinnatum* respectively (Table 48 & Fig. 50).

Morphological studies revealed that in *Westiellopsis prolifica* at 30 ppm, 40 ppm and at 50 ppm of carbofuran the number of heterocysts and branching are frequent than the control. At 60 ppm to 100 ppm, the branching frequency and heterocyst number decreases. The cyanobacterium starts to produce akinetes from 80 ppm to 100 ppm carbofuran. At 70 ppm onwards the green colour of the filaments begins to fade eventhough it can survive. In *Scytonema cincinnatum*, the maximum number of heterocysts and branching are seen at 10 ppm to 30 ppm over the

control. At 40 ppm to100 ppm the branching frequency decreases. At 90 ppm and 100 ppm of carbofuran, the green colour of the filament begins to fade into light green colour.

4.5.2. Discussion

The role of cyanobacteria as biological input in agriculture has been well documented. To protect the crop from the insect pest attack or to control weed population, a large variety of agrochemicals like insecticide and herbicides are widely used. These agrochemicals may also affect the non-target flora in soil including cyanobacteria. Therefore, it is advisable to screen the nitrogen fixing cyanobacteria against these agrochemicals.

The application of pesticides has become a routine practice in controlling pests and disease of rice. The present study was therefore undertaken to know the relative tolerance of cyanobacteria to the insecticide treatments. There are reports that the insecticides are either stimulatory or inhibitory or neutral to cyanobacterial growth and nitrogen fixation (Kaushik and Venkataraman, 1983). The heavy application of toxic agrochemicals especially insecticides, influences the growth and N_2 fixation of rice field cyanobacteria (Venkataraman and Rajalakshmi, 1972). Kar and Singh (1978) reported that carbofuran was commonly used in rice cultivation to stimulate the growth and nitrogen fixation of *Azolla* at low dosages.

Table 38. Effect of carbofuran on growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.23 ± 0.11	0.31 ± 0.10	0.35 ± 0.09	0.42 ± 0.12	0.56 ± 0.09	0.65 ± 0.012
10	0.26± 0.10	0.33 ± 0.08	0.37 ± 0.11	0.44 ± 0.11	0.58 ± 0.09	0.67 ± 0.08
20	0.29 ± 0.12	0.34 ± 0.011	0.39 ± 0.12	0.47 ± 0.08	0.60 ± 0.13	0.69 ± 0.09
30	0.32 ± 0.09	0.36 ± 0.09	0.43 ± 0.15	0.52 ± 0.08	0.63 ± 0.11	0.71 ± 0.08
40	0.36 ± 0.13	0.39 ± 0.09	0.48 ± 0.13	0.59 ± 0.11	0.66 ± 0.12	0.77 ± 0.11
50	0.39 ± 0.011	0.44 ± 0.11	0.52 ± 0.09	0.64 ± 0.09	0.69 ± 0.09	0.88 ± 0.12
60	0.38 ± 0.08	0.46 ± 0.07	0.50 ± 0.13	0.62 ± 0.08	0.65 ± 0.11	0.73 ± 0.08
70	0.35 ± 0.13	0.43 ± 0.13	0.45 ± 0.08	0.53 ± 0.12	0.61 ± 0.08	0.65 ± 0.11
80	0.31 ± 0.14	0.38 ± 0.09	0.40 ± 0.08	0.48 ± 0.11	0.53 ± 0.10	0.59 ± 0.10
90	0.24 ± 0.11	0.33 ± 0.08	0.34 ± 0.11	0.41 ± 0.08	0.45 ± 0.11	0.48± 0.09
100	0.22 ± 0.09	0.24 ± 0.09	0.31 ± 0.12	0.38 ± 0.09	0.41 ± 0.09	0.44 ± 0.10

Values represent mean of three replicates ± S.D.

Table 39. Effect of cabofuran on growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.22 ± 0.10	0.26 ± 0.09	0.29 ± 0.09	0.32 ± 0.08	0.35 ± 0.11	0.38 ± 0.10
10	0.25 ± 0.09	0.30 ± 0.08	0.33 ± 0.12	0.38 ± 0.12	0.42 ± 0.10	0.44 ± 0.011
20	0.27 ± 0.09	0.32 ± 0.11	0.36 ± 0.07	0.41 ± 0.11	0.55 ± 0.08	0.58 ± 0.09
30	0.30 ± 0.11	0.36 ± 0.10	0.39 ± 0.11	0.48 ± 0.09	0.59 ± 0.10	0.64 ± 0.07
40	0.29 ± 0.08	0.34 ± 0.09	0.35 ± 0.10	0.42 ± 0.09	0.53 ± 0.07	0.55 ± 0.11
50	0.24 ± 0.09	0.31 ± 0.12	0.31 ± 0.09	0.35 ±0.08	0.41 ± 0.08	0.48 ± 0.10
60	0.23 ± 0.07	0.28 ± 0.08	0.30 ± 0.10	0.32 ± 0.10	0.35 ± 0.09	0.39 ± 0.12
70	0.22 ± 0.09	0.27 ± 0.07	0.28± 0.11	0.30 ± 0.10	0.33 ± 0.11	0.36 ± 0.07
80	0.21 ± 0.09	0.25 ± 0.10	0.24 ± 0.09	0.26 ± 0.08	0.28 ± 0.07	0.30 ± 0.011
90	0.20 ± 0.08	0.23 ± 0.11	0.23 ± 0.08	0.25 ± 0.07	0.27 ± 0.11	0.21 ± 0.12
100	0.20 ± 0.11	0.22 ± 0.08	0.22 ± 0.12	0.23 ± 0.09	0.23 ± 0.10	0.24 ± 0.11

Values represent mean of three replicates ± S.D.

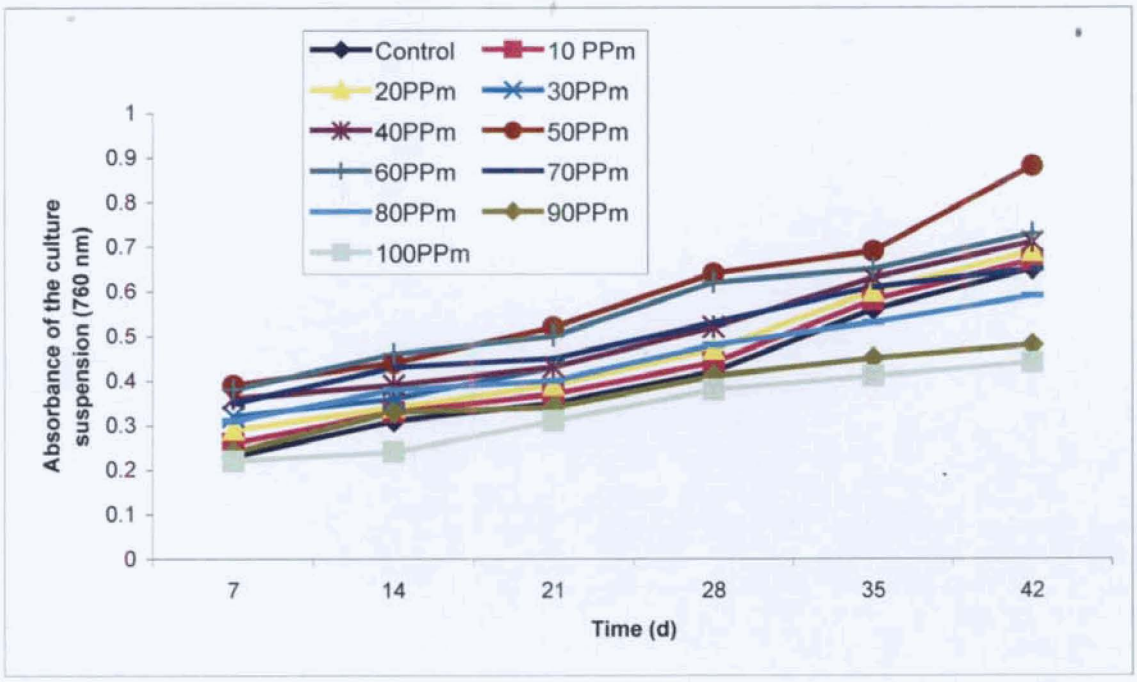


Fig.40 Effect of carbofuran on growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* up to 42 days of incubation.

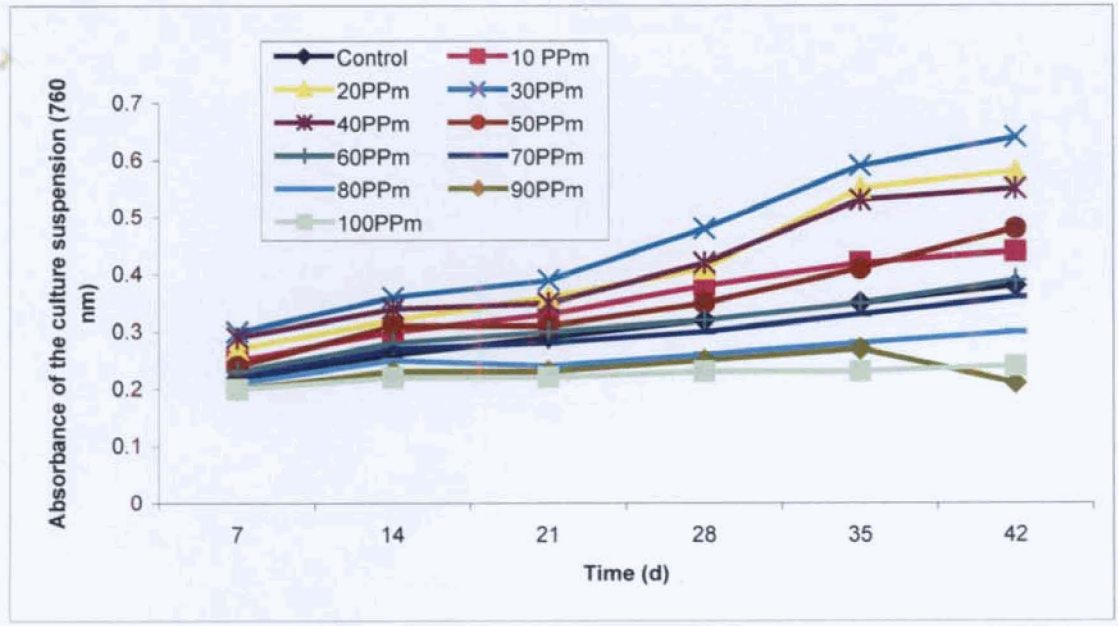


Fig.41 Effect of carbofuran on growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 42 days of incubation.

Table 40. Effect of carbofuran on chlorophyll-a content (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	1.05 ± 0.11	1.24 ± 0.09	2.86 ± 0.06	3.45 ± 0.09	3.88 ± 0.06	4.22 ± 0.08
10	1.21 ± 0.07	1.26 ± 0.09	3.31 ± 0.05	3.52 ± 0.08	3.63 ± 0.09	4.11 ± 0.07
20	1.24 ± 0.09	1.28 ± 0.08	3.52 ± 0.06	3.64 ± 0.08	3.75 ± 0.06	4.26 ± 0.09
30	1.33 ± 0.08	1.57 ± 0.08	3.42 ± 0.07	3.73 ± 0.06	4.04 ± 0.08	4.45 ± 0.06
40	1.48 ± 0.12	1.78 ± 0.06	3.97 ± 0.06	3.86 ± 0.07	4.41 ± 0.09	4.72 ± 0.08
50	1.62 ± 0.06	2.05 ± 0.09	4.01 ± 0.08	4.42 ± 0.06	4.83 ± 0.09	4.11 ± 0.05
60	1.57 ± 0.07	1.81 ± 0.07	3.71 ± 0.12	4.14 ± 0.06	4.57 ± 0.06	4.83 ± 0.06
70	1.51 ± 0.08	1.66 ± 0.05	3.44 ± 0.11	3.56 ± 0.05	3.59 ± 0.07	4.61 ± 0.05
80	1.19 ± 0.07	1.42 ± 0.07	2.33 ± 0.09	2.38 ± 0.05	2.58 ± 0.08	3.15 ± 0.08
90	1.11 ± 0.06	1.21 ± 0.10	2.24 ± 0.08	2.29 ± 0.06	2.33 ± 0.09	2.38 ± 0.07
100	1.01 ± 0.08	1.14 ± 0.08	1.17 ± 0.10	1.20 ± 0.08	1.24 ± 0.07	1.29 ± 0.09

Values represent mean of three replicates ± S.D.

Table 41. Effect of carbofuran on chlorophyll-a content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	1.02 ± 0.09	1.07 ± 0.07	2.12 ± 0.05	2.53 ± 0.07	3.16 ± 0.08	3.56 ± 0.06
10	1.04 ± 0.06	1.06 ± 0.08	2.21 ± 0.06	2.61 ± 0.08	3.26 ± 0.07	3.41 ± 0.08
20	1.06 ± 0.08	1.09 ± 0.07	2.42 ± 0.08	2.66 ± 0.06	3.32 ± 0.07	3.72 ± 0.07
30	1.12 ± 0.06	1.13 ± 0.09	2.68 ± 0.06	2.92 ± 0.05	3.53 ± 0.05	3.93 ± 0.06
40	1.16 ± 0.09	1.21 ± 0.08	2.39 ± 0.05	2.64 ± 0.05	3.26 ± 0.04	3.58 ± 0.05
50	1.15 ± 0.08	1.19 ± 0.07	2.32 ± 0.06	2.61 ± 0.06	3.22 ± 0.07	3.51 ± 0.05
60	1.07 ± 0.05	1.11 ± 0.06	2.27 ± 0.07	2.68 ± 0.07	3.18 ± 0.06	3.46 ± 0.09
70	1.04 ± 0.06	1.08 ± 0.04	2.23 ± 0.08	2.64 ± 0.09	3.11 ± 0.08	3.43 ± 0.05
80	1.03 ± 0.07	1.06 ± 0.06	2.13 ± 0.08	2.51 ± 0.06	3.06 ± 0.05	3.37 ± 0.08
90	1.02 ± 0.09	1.03 ± 0.07	1.07 ± 0.05	1.08 ± 0.06	1.09 ± 0.07	1.09 ± 0.07
100	1.01 ± 0.08	1.02 ± 0.05	1.03 ± 0.07	1.03 ± 0.08	1.04 ± 0.06	1.04 ± 0.09

Values represent mean of three replicates ± S.D.

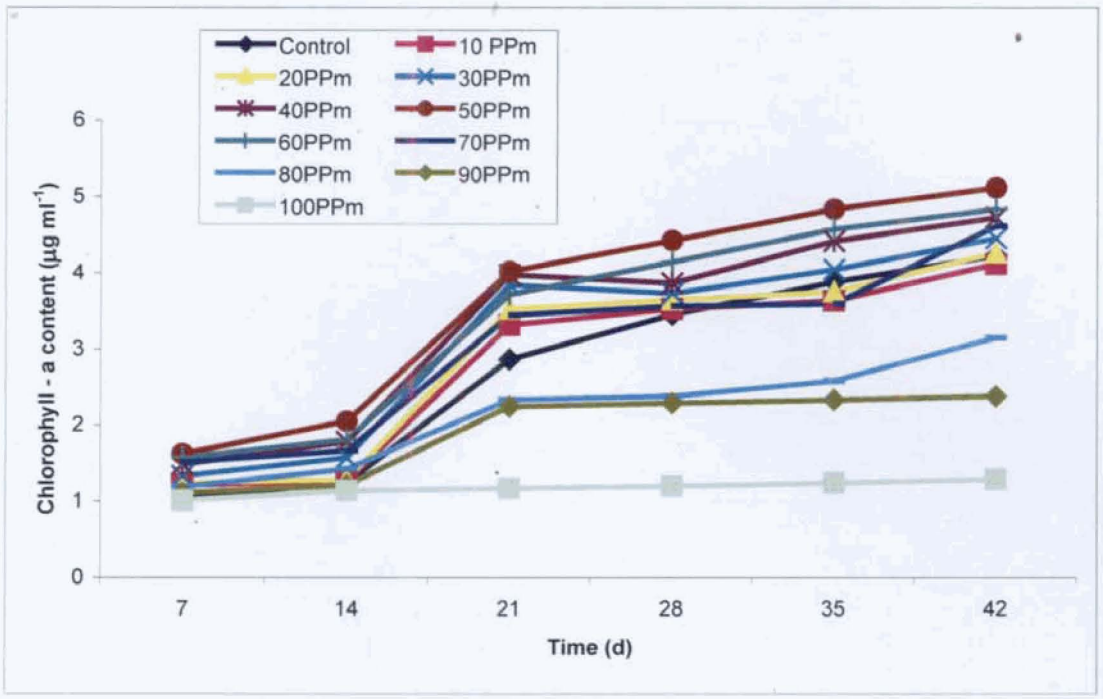


Fig.42 Effect of carbofuran on chlorophyll-a content of *Westiellopsis prolifica* up to 42 days of incubation.

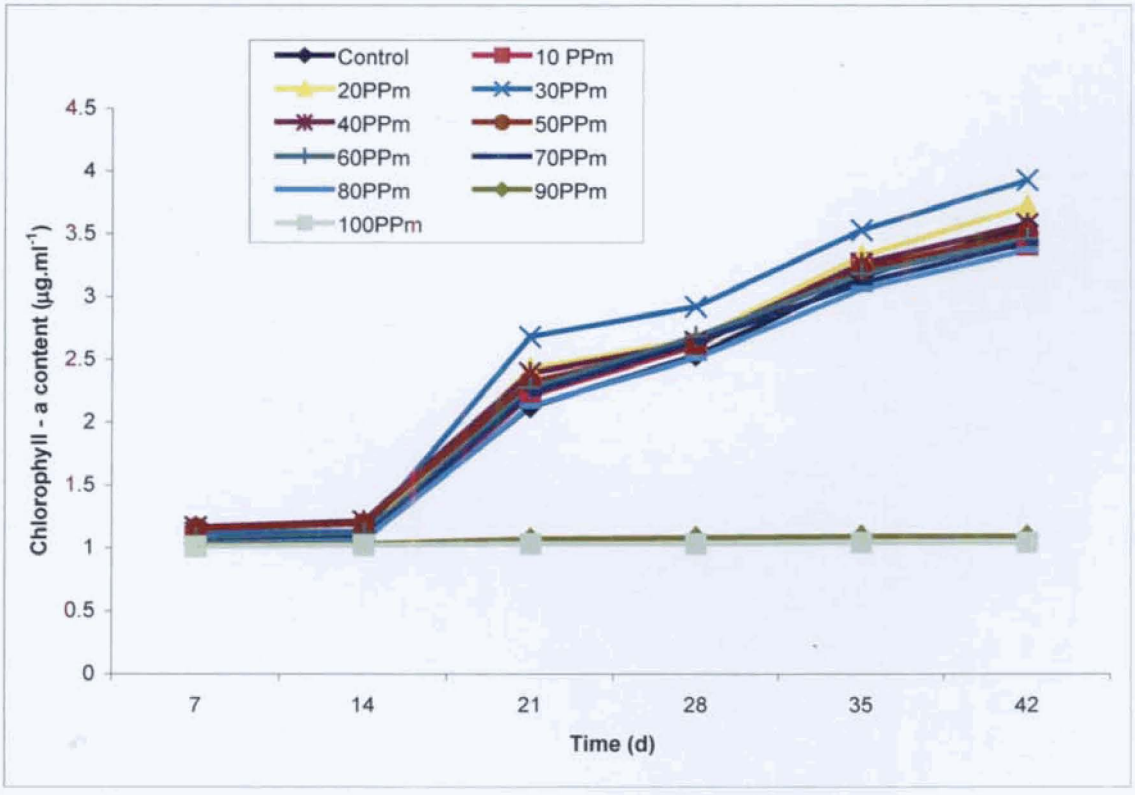


Fig.43 Effect of carbofuran on chlorophyll-a content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 42. Effect of carbofuran on carotene content (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C for 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.673 ± 0.03	0.685 ± 0.05	0.721 ± 0.05	0.739± 0.06	0.753 ± 0.06	0.764 ± 0.04
10	0.692 ± 0.04	0.723 ± 0.06	0.784 ± 0.06	0.816 ± 0.07	0.821± 0.05	0.845 ± 0.05
20	0.791 ± 0.02	0.801 ± 0.04	0.823 ± 0.05	0.844 ± 0.07	0.862 ± 0.02	0.875 ± 0.05
30	0.798 ± 0.03	0.816 ± 0.03	0.874 ± 0.03	0.881 ± 0.02	0.895 ± 0.02	0.912 ± 0.07
40	0.816 ± 0.05	0.832 ± 0.07	0.889 ± 0.04	0.902 ± 0.03	0.923 ± 0.04	0.936 ± 0.03
50	0.839 ± 0.04	0.863 ± 0.04	0.916 ± 0.07	0.936 ± 0.03	0.951 ± 0.03	0.967 ± 0.02
60	0.815 ± 0.03	0.856 ± 0.03	0.909 ± 0.05	0.921 ± 0.07	0.930 ± 0.04	0.951 ± 0.03
70	0.776 ± 0.06	0.792 ± 0.06	0.816 ± 0.05	0.824 ± 0.04	0.873 ± 0.03	0.893 ± 0.06
80	0.671 ± 0.03	0.680 ± 0.06	0.711 ± 0.03	0.726 ± 0.03	0.732 ± 0.06	0.756 ± 0.02
90	0.675 ± 0.04	0.679 ± 0.05	0.702 ± 0.06	0.723 ± 0.06	0.745 ± 0.03	0.762 ± 0.04
100	0.623 ± 0.06	0.628 ± 0.04	0.632 ± 0.05	0.641 ± 0.05	0.646 ± 0.04	0.652 ± 0.06

Values represent mean of three replicates ± S.D.

Table 43. Effect of carbofuran on carotene content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C for 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.143 ± 0.09	0.152 ± 0.08	0.171 ± 0.07	0.223 ± 0.06	0.306 ± 0.05	0.311 ± 0.06
10	0.162 ± 0.06	0.172 ± 0.07	0.186 ± 0.09	0.213 ± 0.07	0.312 ± 0.06	0.324 ± 0.08
20	0.189 ± 0.04	0.196 ± 0.04	0.214 ± 0.06	0.236 ± 0.05	0.315 ± 0.06	0.356 ± 0.07
30	0.192 ± 0.04	0.223 ± 0.05	0.258 ± 0.07	0.295 ± 0.04	0.364 ± 0.07	0.402 ± 0.08
40	0.181 ± 0.06	0.194 ± 0.07	0.211 ± 0.08	0.231 ± 0.04	0.311 ± 0.06	0.349 ± 0.09
50	0.173 ± 0.05	0.191 ± 0.08	0.209 ± 0.05	0.227 ± 0.06	0.306 ± 0.05	0.342 ± 0.06
60	0.168 ± 0.09	0.186 ± 0.05	0.206 ± 0.05	0.221 ± 0.08	0.301 ± 0.04	0. 316 ± 0.05
70	0.164 ± 0.07	0.183 ± 0.06	0.204 ± 0.06	0.219 ± 0.07	0.295 ± 0.06	0.303 ± 0.04
80	0.161 ± 0.07	0.171 ± 0.08	0.188 ± 0.08	0.215 ± 0.06	0.292 ± 0.07	0.298 ± 0.05
90	0.141 ± 0.06	0.144 ± 0.09	0.149 ± 0.06	0.151 ± 0.07	0.154 ± 0.06	0.159 ± 0.07
100	0.138 ± 0.07	0.139 ± 0.06	0.139 ± 0.08	0.141 ± 0.06	0.142 ± 0.07	0.142 ± 0.08

Values represent mean of three replicates ± S.D.

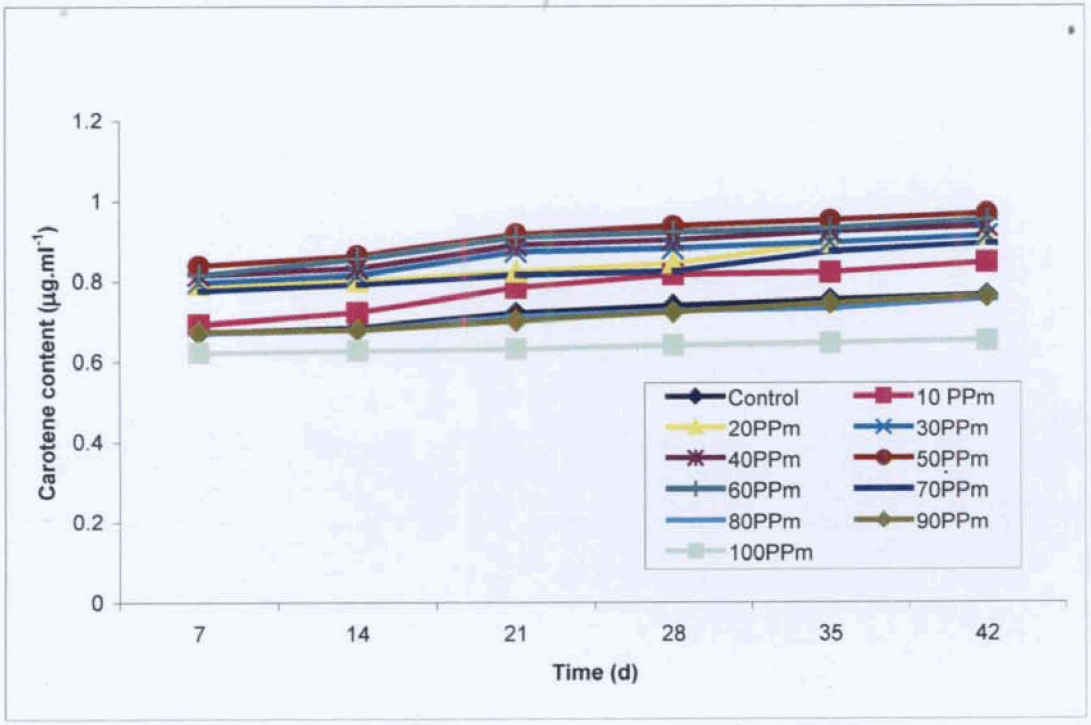


Fig.44 Effect of carbofuran on carotene content of *Westiellopsis prolifica* up to 42 days of incubation.

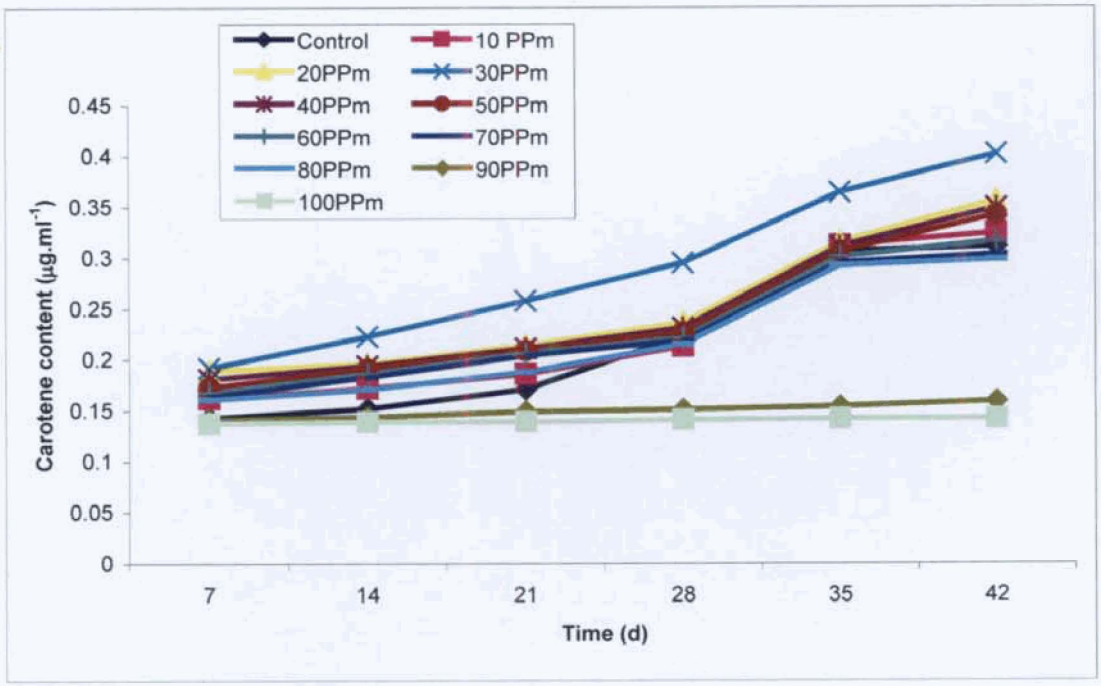


Fig.45 Effect of carbofuran on carotene content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 44. Effect of carbofuran on the protein content (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	38.88 ± 0.07	44.36 ± 0.05	52.17 ± 0.09	59.72 ± 0.05	65.41 ± 0.06	76.97 ± 0.04
10	29.37 ± 0.06	36.28± 0.07	42.11 ± 0.06	53.62 ± 0.07	61.09 ± 0.08	72.16 ± 0.09
20	30.55 ± 0.05	42.92 ± 0.04	53.84 ± 0.06	63.33 ± 0.03	73.44 ± 0.07	82.31 ± 0.03
30	33.46 ± 0.06	45.73 ± 0.08	58.39 ± 0.05	65.14 ± 0.07	79.37 ± 0.06	88.46 ± 0.03
40	37.91 ± 0.04	52.16 ± 0.06	61.37 ± 0.04	67.36 ± 0.07	82.12 ± 0.08	92.34 ± 0.05
50	34.58 ± 0.09	56.34 ± 0.05	65.19 ± 0.05	68.11 ± 0.05	92.19 ± 0.07	102.37 ± 0.03
60	32.30 ± 0.06	40.37 ± 0.07	51.94 ± 0.06	62.51 ± 0.08	83.17 ± 0.09	85.75 ± 0.04
70	30.17 ± 0.05	38.82 ± 0.09	45.41 ± 0.05	56.37 ± 0.06	77.77 ± 0.08	78.31 ± 0.06
80	29.34 ± 0.03	36.76 ± 0.07	43.39 ± 0.09	50.32 ± 0.07	73.49 ± 0.05	74.78 ± 0.06
90	21.16 ± 0.05	24.22 ± 0.08	28.36 ± 0.09	31.72 ± 0.08	35.86 ± 0.04	39.92 ± 0.05
100	19.11± 0.08	20.31 ± 0.06	24.25 ± 0.07	29.46 ± 0.05	31.71 ± 0.06	35.67 ± 0.04

Values represent mean of three replicates ± S.D.

Table 45. Effect of carbofuran on the protein content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 42 days of incubation at 25 ± 1°C with 12h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	26.37 ± 0.09	31.11 ± 0.11	35.73 ± 0.09	41.09 ± 0.08	44.16 ± 0.09	45.73 ± 0.08
10	22.41 ± 0.08	28.62 ± 0.09	31.64 ± 0.08	35.37 ± 0.07	39.72 ± 0.06	41.68 ± 0.07
20	24.46 ± 0.11	32.17 ± 0.11	36.41 ± 0.08	42.97 ± 0.09	45.23 ± 0.08	46.73 ± 0.09
30	23.93 ± 0.12	34.26 ± 0.09	39.39 ± 0.07	45.28 ± 0.06	49.36 ± 0.06	54.62 ± 0.07
40	23.67 ± 0.08	31.98 ± 0.08	35.44 ± 0.07	41.86 ± 0.11	44.96 ± 0.09	46.11 ± 0.09
50	22.35 ± 0.07	31.62 ± 0.07	35.03 ± 0.09	41.15 ± 0.09	44.27 ± 0.09	45.71 ± 0.11
60	22.16 ± 0.09	31.43 ± 0.09	34.78 ± 0.08	40.37 ± 0.08	43.81 ± 0.08	45.43 ± 0.09
70	21.01 ± 0.09	31.19 ± 0.07	34.12 ± 0.011	39.29 ± 0.09	43.16 ± 0.08	44.29 ± 0.08
80	20.23 ± 0.11	30.16 ± 0.07	33.61 ± 0.09	39.01 ± 0.08	42.32 ± 0.07	43.72 ± 0.07
90	18.61 ± 0.09	21.73 ± 0.06	26.37 ± 0.08	29.17± 0.07	31.63 ± 0.06	35.28 ± 0.06
100	15.32 ± 0.08	15.77 ± 0.07	16.42 ± 0.06	17.32 ± 0.06	19.72 ± 0.07	21.16 ± 0.08

Values represent mean of three replicates ± S.D.

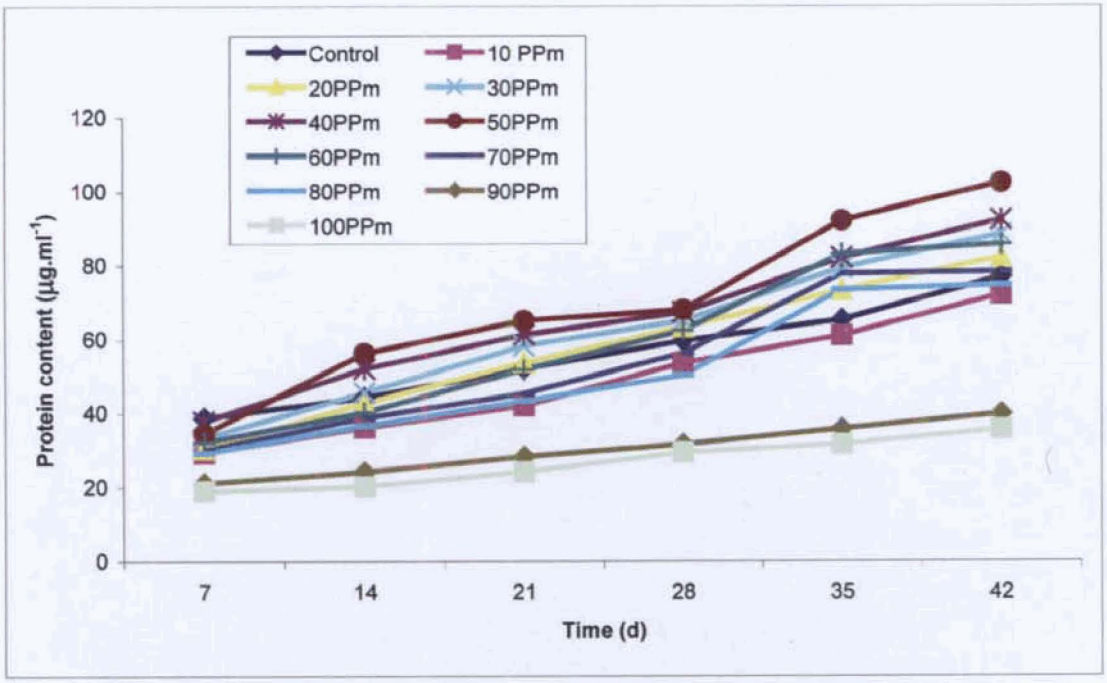


Fig.46 Effect of carbofuran on protein content of *Westiellopsis prolifica* up to 42 days of incubation.

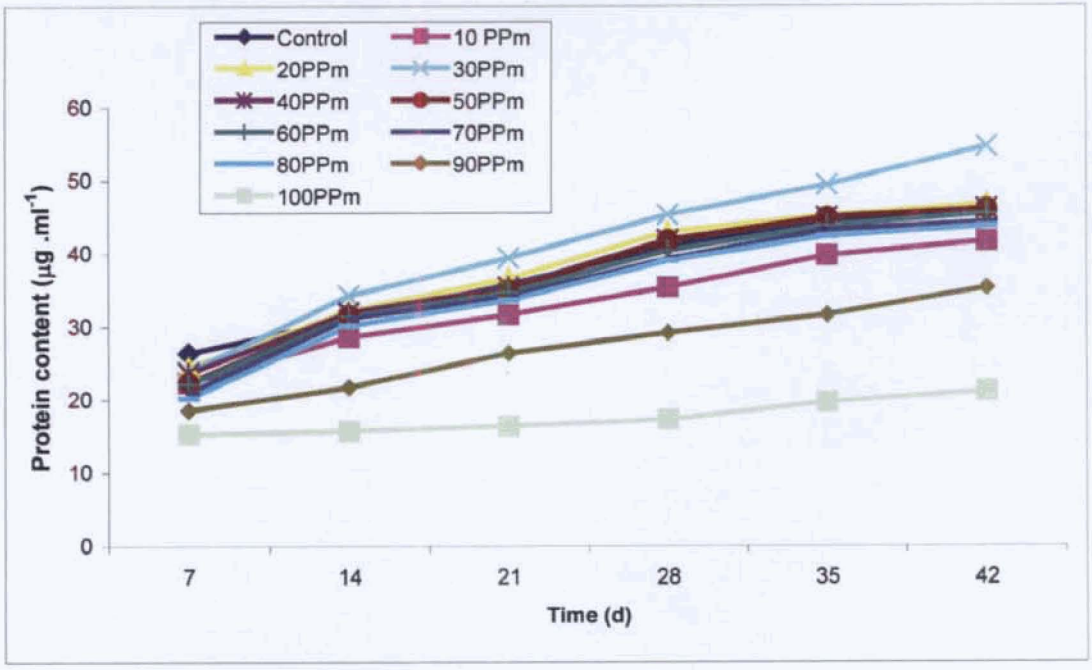


Fig.47 Effect of carbofuran on protein content of *Scytonema cincinnatum* up to 42 days of incubation.

Table 46. Effect of carbofuran on phycobiliproteins of *Westiellopsis prolifica* on 42nd day of incubation

Concentration (ppm)	Control	10	20	30	40	50	60	70	80	90	100
PC	25.86 ± 0.04	25.91 ± 0.05	26.33 ± 0.08	27.01 ±0.09	27.83 ± 0.05	28.68 ± 0.07	28.45 ±0.06	27.12 ±0.08	26.54 ±0.07	25.19 ±0.08	24.08 ± 0.09
APC	36.11 ± 0.06	36.52 ± 0.04	36.94 ± 0.05	37.42± 0.04	37.94 ± 0.06	38.45 ±0.08	38.13 ±0.07	36.26 ±0.04	35.42 ±0.05	34.41 ±0.06	35.21 ± 0.05
PE	2.32 ±0.03	2.87 ± 0.07	2.87 ± 0.04	2.94 ±0.03	3.16 ±0.05	3.81 ±0.05	3.43 ±0.06	3.06 ±0.05	2.26 ±0.07	2.14 ±0.05	2.07 ±0.04

Values represent mean of three replicates ± S.D.

Table 47. Effect of carbofuran on phycobiliproteins of *Scytonema cincinnatum* on 42nd day of incubation

Concentration (ppm)	Control	10	20	30	40	50	60	70	80	90	100
PC	25.98 ±0.09	26.57 ±0.08	27.12 ±0.10	27.98 ±0.09	27.01 ±0.07	26.23 ±0.09	25.14 ±0.10	24.45 ±0.09	24.03 ±0.08	23.54 ±0.09	23.21 ±0.09
APC	22.84 ± 0.05	23.41 ± 0.07	24.53 ± 0.06	24.83 ± 0.08	24.23 ±0.06	23.89 ± 0.10	22.36 ± 0.08	23.21 ± 0.06	22.86 ± 0.09	22.24 ± 0.08	22.01 ± 0.08
PE	1.723 ± 0.06	1.758 ± 0.07	1.826 ± 0.09	1.893 ± 0.06	1.811 ± 0.07	1.721 ± 0.06	1.718 ± 0.09	1.681 ± 0.05	1.642 ± 0.10	1.623 ± 0.07	1.603 ± 0.06

Values represent mean of three replicates ± S.D.

Table 48. Effect of carbofuran on ammonia excretion of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation

Concentration (ppm)	Control	10	20	30	40	50	60	70	80	90	100
Westiellopsis prolifica	15.01 ± 0.06	22.36 ± 0.08	26.84 ± 0.05	30.39 ± 0.06	28.31 ± 0.05	27.42 ± 0.08	25.68 ± 0.05	18.11 ± 0.04	15.46 ± 0.07	15.01 ± 0.06	14.63 ± 0.05
Scytonema cincinnatum	18.54 ± 0.07	20.81 ± 0.09	24.16 ± 0.06	28.72 ± 0.07	25.34 ± 0.08	23.63 ± 0.07	21.72 ± 0.06	17.38 ± 0.05	15.21 ± 0.08	14.84 ± 0.05	13.16 ± 0.06

Values represent mean of three replicates ± S.D.

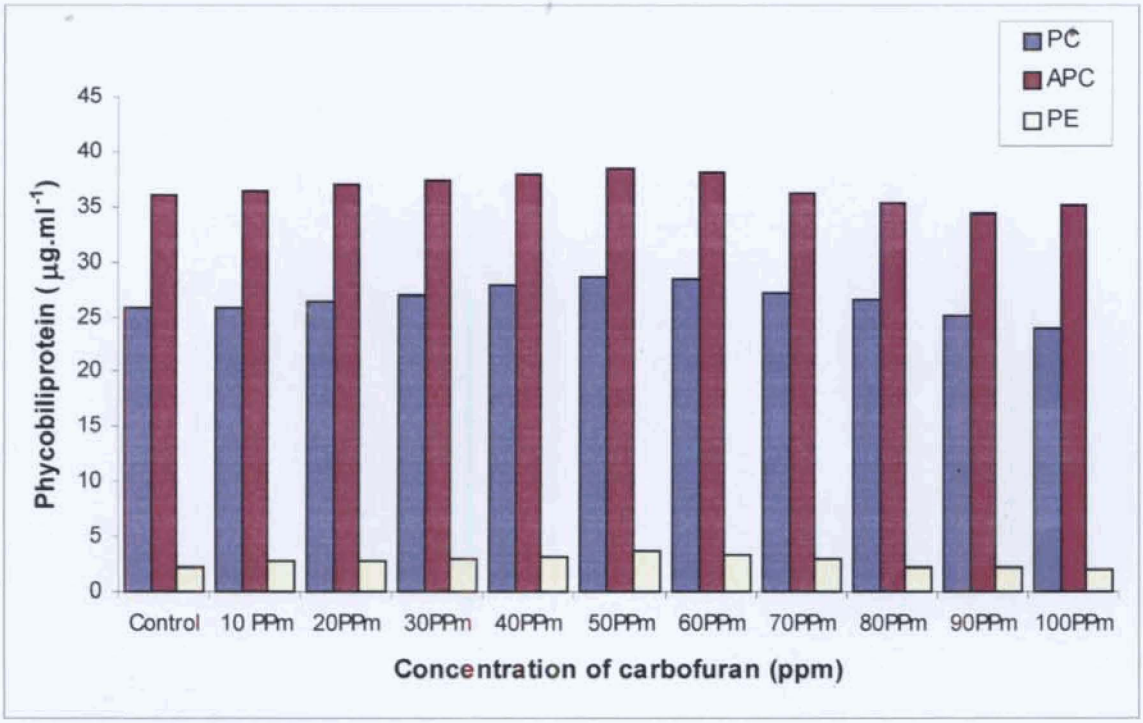


Fig.48 Effect of carbofuran on phycobiliproteins of *Westiellopsis prolifica* on 42nd day of incubation.

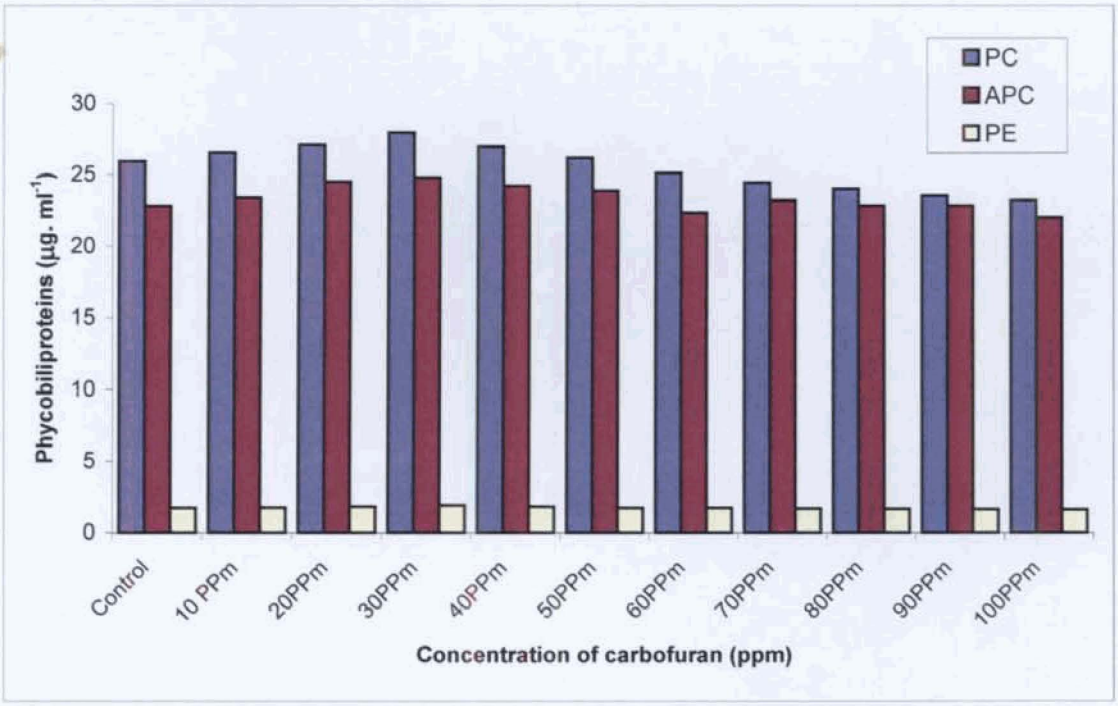


Fig.49 Effect of carbofuran on phycobiliproteins of *Scytonema cincinnatum* on 42nd day of incubation.

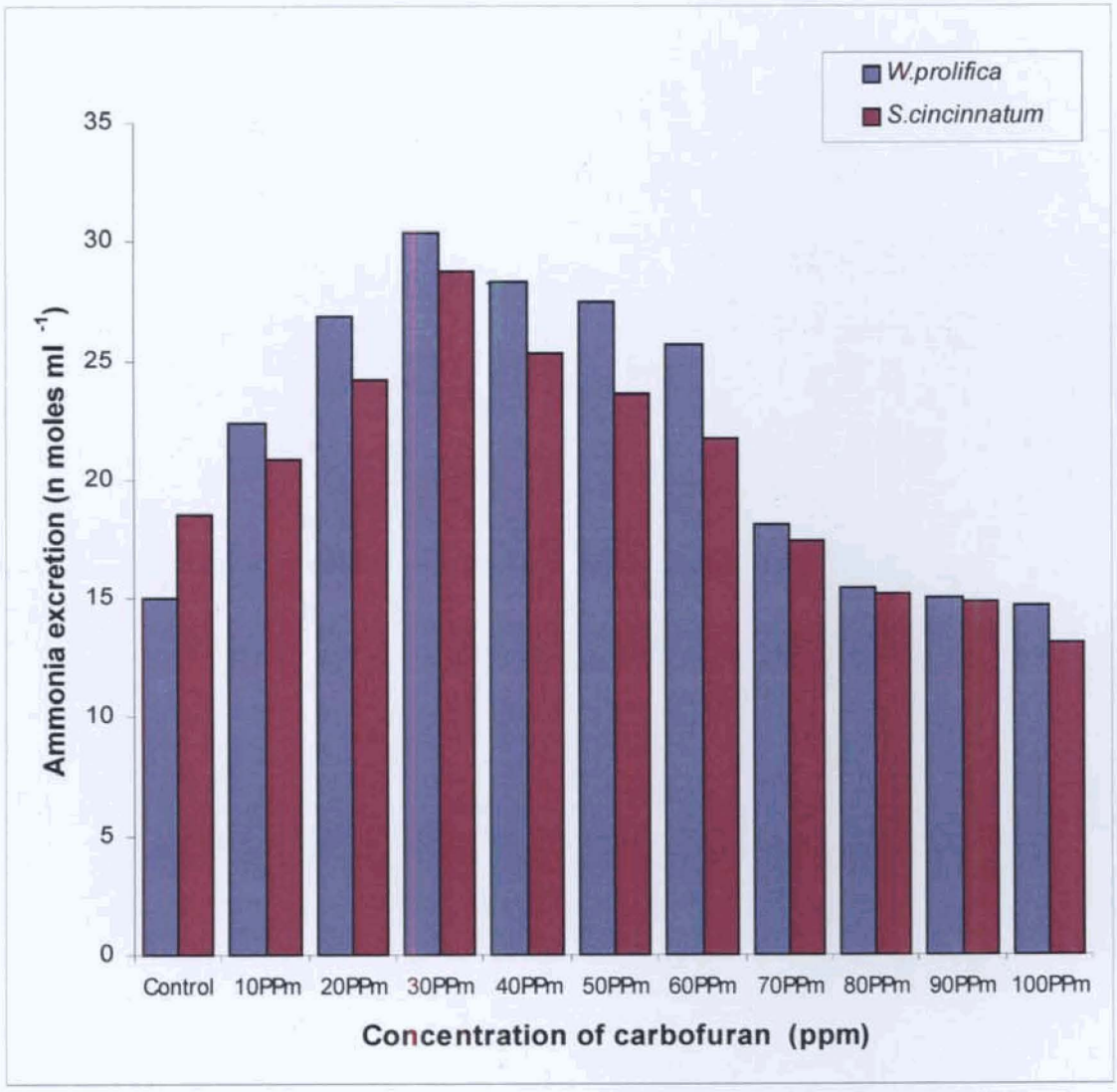


Fig.50 Effect of carbofuran on ammonia excretion of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation.

According to the present investigation, *Westiellopsis prolifica* and *Scytonema cincinnatum* showed a varying response to the insecticide carbofuran. The production of pigments, proteins and ammonia excretion in *Westiellopsis prolifica* are greater in carbofuran treated cyanobacterial cultures compared to the control. This was in accordance to the findings of Uma and Kannaiyan (1998). There are reports that cyanobacteria can tolerate carbofuran up to a level of 100 ppm in *Calothrix braunii* 367, *Hapalosiphon fontinalis* 363, *Westiellopsis prolifica* 365 (Kaushik and Venkataraman, 1983). In *Scytonema cincinnatum* there was a slight reduction in chlorophyll-a and carotene content than control at higher concentrations of carbofuran after 28 days of incubation. This is because the insecticides affect differentially on the growth and different genera respond differently and there was an inter and intra specific strain variation.

According to Tirol *et al.* (1981), the nitrifying activity increased with increasing rate of carbofuran of 10-100 ppm. There are positive reports that carbofuran did not inhibit growth, chlorophyll-a content and ammonia production (Ghosh and Saha, 1988; Megharaj *et al.*, 1988, 1989). More over, the presence of nutrients affects the toxicity of carbofuran (Kar and Singh, 1978). The toxicity of the insecticide decreased with incubation period which suggests the detoxification of insecticide by cyanobacteria.

Since carbofuran is inexpensive and easily available insecticide commonly used in rice fields to control insect pests, it can be employed to

serve dual purpose of an insecticide and a stimulant of ammonia release by cyanobacteria in rice fields.

4.6. EFFECT OF NEEM ON GROWTH CHARACTERISTICS OF CYANOBACTERIA *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM*

4.6.1. Results

Growth response (absorbance of the culture suspension at 760 nm) as well as protein, chlorophyll-a content, carotene content, phycobiliprotein content and ammonia excretion of *Westiellopsis prolifica* and *Scytonema cincinnatum* at different concentrations of neem was presented in the Tables (49-51). The organisms were grown at 20, 40, 60, 80, 100, 200, 300 and 400 ppm of neem for a period of 42 days. Of the two species, *Westiellopsis prolifica* showed higher growth, pigment content, protein content and ammonia excretion in all concentration of neem with maximum at 300 ppm over the control up to 42 days of growth. In contrary, *Scytonema cincinnatum* showed higher growth at control and growth will be lesser at different neem concentrations.

The growth response of *Westiellopsis prolifica* and *Scytonema cincinnatum* was presented in the Tables (49, 50 & Fig. 51, 52) respectively. In *Westiellopsis prolifica* growth rate increased tremendously after 14 days of incubation. It showed that *Westiellopsis prolifica* takes about two weeks time to adapt with new environment. The growth rate increased up to 400 ppm of

neem with maximum at 300 ppm. At 400 ppm the growth was slightly decreased. In *Scytonema cincinnatum* growth rate was higher for control and it decreased up to 400 ppm of neem concentration. In *Westiellopsis prolifica*, chlorophyll-a, carotene and protein content was maximum at 300 ppm of neem and was presented in the Tables (51, 52, 53 & Fig. 53, 54, 55).

Scytonema cincinnatum can tolerate neem solution up to two weeks, and later the greencolour of the filaments fades off and the filaments showed signs of disintegration. The chlorophyll-a and carotene content was maximum for the control and it decreased considerably at higher neem concentrations (Tables 54, 55 & Fig. 56, 57). But the protein content showed a slight increase over the control at higher neem concentrations (Table 56 & Fig. 58).

The phycobiliprotein content increased considerably at higher neem concentrations and was maximum at 400 ppm of neem for *Westiellopsis prolifica* than the control (Table 57 & Fig. 59) while in *Scytonema cincinnatum*, very minute increase was noticed at higher concentration of neem over the control and it gets levelled off at 300 and 400 ppm (Table 58 & Fig. 60). Ammonia excretion was maximum at 300 ppm for *Westiellopsis prolifica* and in control for *Scytonema cincinnatum* on 14^{th} day of incubation (Table 59 & Fig. 61). The heterocyst frequency increased slightly at higher neem concentrations in *Westiellopsis prolifica* while in *Scytonema cincinnatum* it reduced to half, compared to the control, at higher neem concentrations (Table 60 & Fig. 62).

Table 49. Effect of neem on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ±1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.24 ± 0.19	0.28 ± 0.15	0.34 ± 0.15	0.39 ± 0.16	0.54 ± 0.15	0.65 ± 0.14
20	0.24 ± 0.16	0.29 ± 0.16	0.44 ± 0.19	0.48 ± 0.11	0.56 ± 0.19	0.66 ± 0.16
40	0.25 ± 0.18	0.31 ± 0.15	0.46 ± 0.12	0.51 ± 0.13	0.59 ± 0.16	0.68 ± 0.17
60	0.27 ± 0.16	0.34 ± 0.14	0.51 ± 0.11	0.59 ± 0.15	0.65 ± 0.10	0.70± 0.16
80	0.28 ± 0.15	0.38 ± 0.19	0.56 ± 0.18	0.60 ± 0.13	0.68 ± 0.11	0.72 ± 0.15
100	0.29 ± 0.14	0.41 ± 0.17	0.55 ± 0.19	0.62 ± 0.14	0.70 ± 0.12	0.74± 0.14
200	0.30 ± 0.19	0.42 ± 0.16	0.59 ± 0.15	0.65 ± 0.16	0.72 ± 0.12	0.80 ± 0.13
300	0.32 ± 0.15	0.48 ± 0.14	0.61± 0.11	0.68 ± 0.13	0.74 ± 0.15	0.82 ± 0.14
400	0.31 ± 0.11	0.45 ± 0.12	0.57 ± 0.10	0.66 ± 0.11	0.73 ± 0.16	0.79 ± 0.15

Values represent mean of three replicates ± S.D.

Table 50. Effect of neem on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* on 14th day of incubation. Cultures were incubated at 25± 1°C with 12h light/dark cycle

Control	Concentration (ppm)							
	20	40	60	80	100	200	300	400
0.32 ± 0.16	0.30 ± 0.11	0.29 ± 0.14	0.28 ± 0.13	0.27 ± 0.12	0.26 ± 0.13	0.25 ± 0.13	0.22 ± 0.15	0.20 ± 0.11

Values represent mean of three replicates ± S.D.

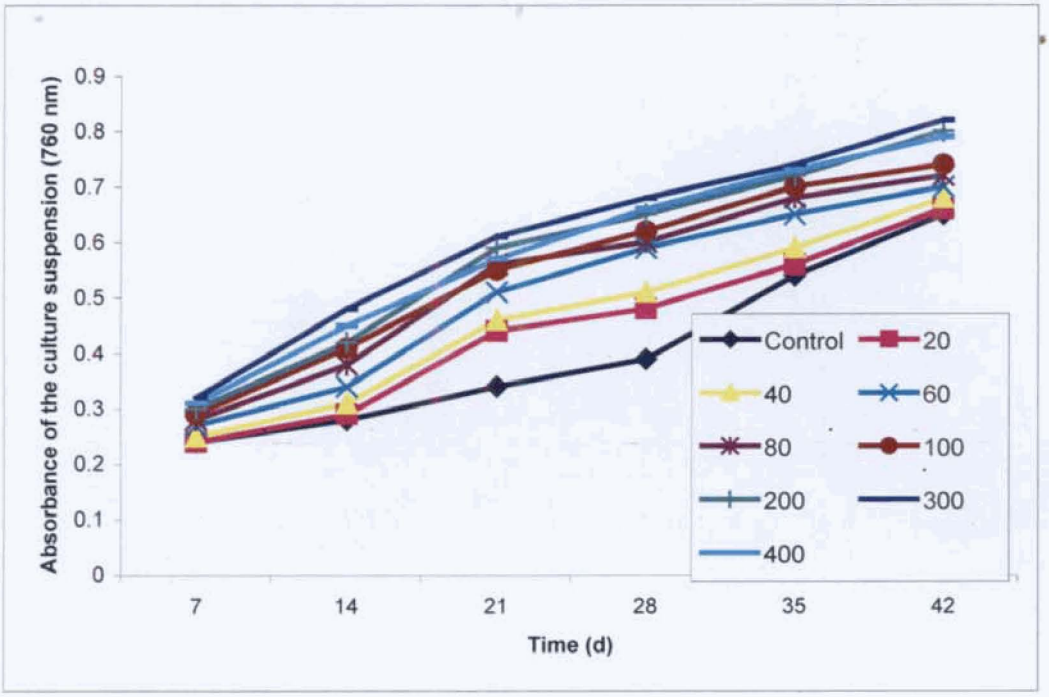


Fig.51 Effect of neem on growth (absorbance of the culture suspension at 760nm) of *Westiellopsis prolifica.*

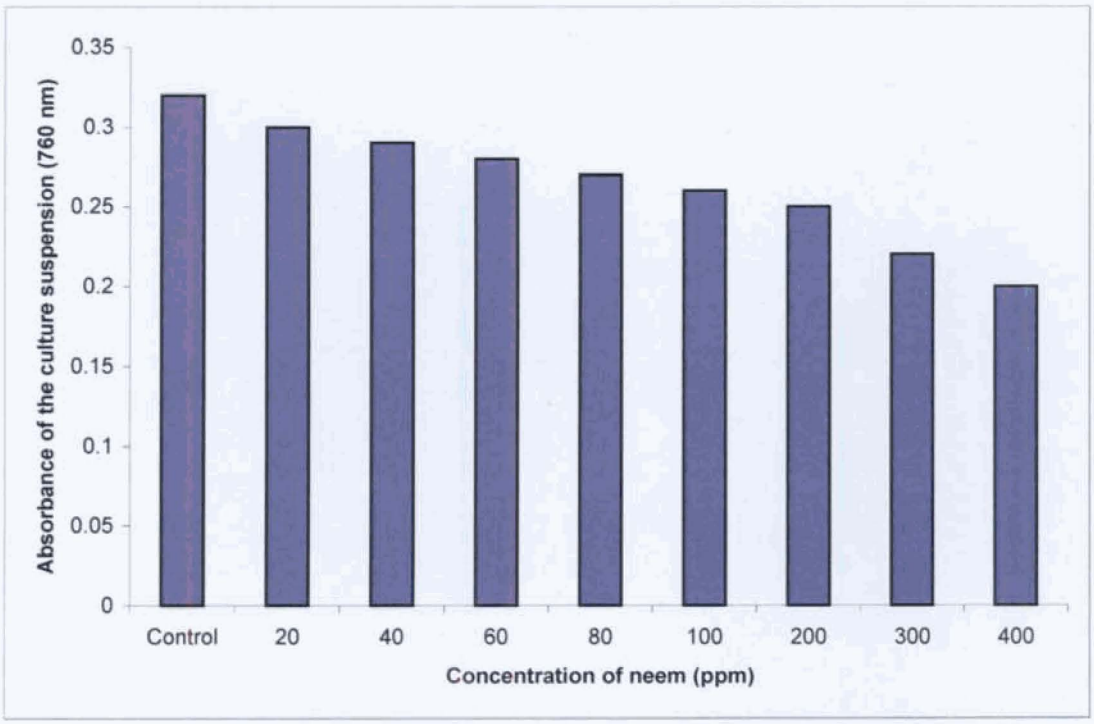


Fig.52 Effect of neem on the growth (absorbance of the culture suspension at 760nm) of *Scytonema cincinnatum* on 14th day of incubation.

Table 51. Effect of neem on chlorophyll –a content (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.99 ± 0.04	1.63 ± 0.07	2.95 ± 0.05	3.71 ± 0.04	0.89 ± 0.06	4.21 ± 0.05
20	1.11 ± 0.03	1.87 ± 0.05	3.13 ± 0.07	3.58 ± 0.05	2.93 ± 0.07	4.67 ± 0.07
40	1.19 ± 0.05	1.92 ± 0.04	3.31 ± 0.06	3.75 ± 0.04	3.93 ± 0.04	4.86 ± 0.04
60	1.28 ± 0.04	2.18 ± 0.06	3.83 ± 0.05	3.88 ± 0.03	3.97 ± 0.05	4.99 ± 0.06
80	1.31 ± 0.06	2.42 ± 0.05	3.89 ± 0.03	3.96 ± 0.05	4.03 ± 0.05	5.12 ± 0.05
100	1.34 ± 0.08	3.12 ± 0.05	4.19 ± 0.06	4.87 ± 0.03	5.21 ± 0.03	5.58 ± 0.07
200	1.58 ± 0.06	3.86 ± 0.06	4.85 ± 0.05	5.43 ± 0.04	6.37 ± 0.04	6.13 ± 0.03
300	1.99 ± 0.03	3.93 ± 0.07	4.95 ± 0.06	5.67 ± 0.05	6.98 ± 0.05	6.82 ± 0.04
400	1.52 ± 0.09	3.75 ± 0.07	4.81 ± 0.08	5.42 ± 0.07	5.90 ± 0.04	6.03 ± 0.07

Values represent mean of three replicates ± S.D.

Table 52. Effect of neem on carotene content (μg. ml^{-1} culture) of *Westiellopsis prolifica* upto 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	0.416 ± 0.06	0.432 ± 0.08	0.516 ± 0.09	0.694 ± 0.11	0.836 ± 0.09	0.887 ± 0.08
20	0.421 ± 0.09	0.457 ± -0.09	0.611 ± 0.11	0.722 ± 0.09	0.892 ± 0.05	0.901 ± 0.09
40	0.433 ± 0.11	0.483 ± 0.08	0.627 ± 0.07	0.731 ± 0.06	0.899 ± 0.06	0.927 ± 0.08
60	0.451 ± 0.012	0.501 ± 0.07	0.633 ± 0.08	0.752 ± 0.08	0.932 ± 0.06	0.945 ± 0.07
80	0.463 ± 0.08	0.524 ± 0.09	0.691 ± 0.07	0.795 ± 0.07	0.973 ± 0.07	0.987 ± 0.06
100	0.476 ± 0.09	0.563 ± 0.11	0.725 ± 0.09	0.917 ± 0.08	1.380 ± 0.08	1.431 ± 0.07
200	0.561 ± 0.08	0.684 ± 0.09	0.789 ± 0.07	0.954 ± 0.09	1.411 ± 0.09	1.522 ± 0.08
300	0.512 ± 0.07	0.693 ± 0.12	0.796 ± 0.06	0.989 ± 0.12	1.510 ± 0.07	1.580 ± 0.09
400	0.510 ± 0.06	0.690 ± 0.11	0.791 ± 0.04	0.982 ± 0.09	1.423 ± 0.06	1.641 ± 0.06

Values represent mean of three replicates ± S.D.

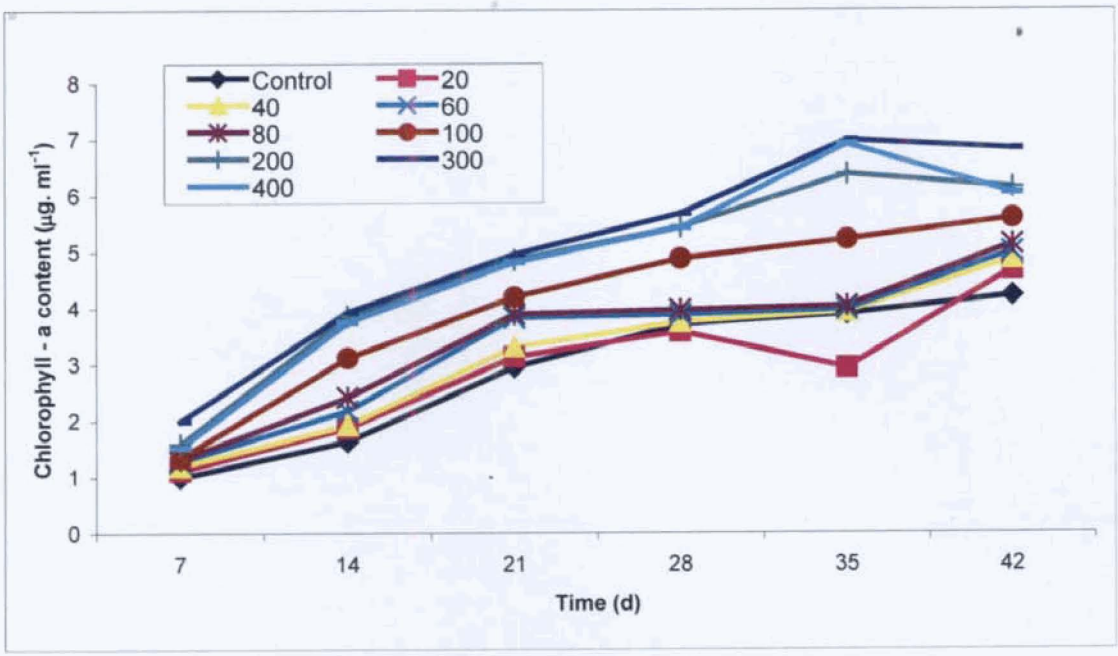


Fig.53 Effect of neem on chlorophyll-a content of *Westiellopsis prolifica* up to 42 days of incubation.

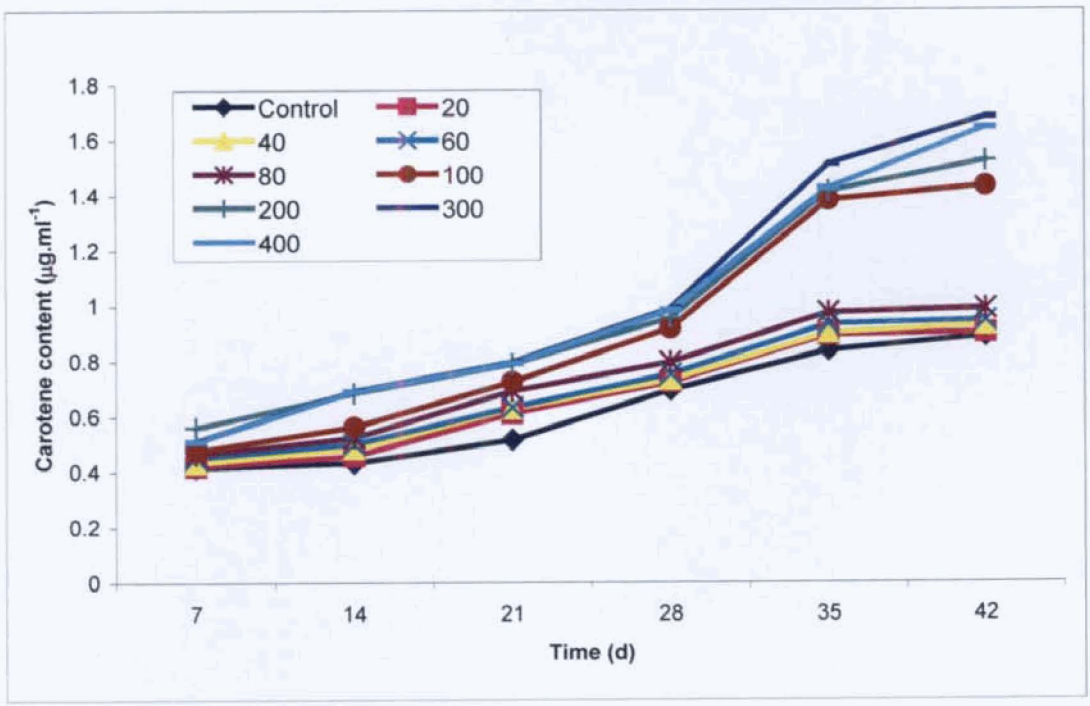


Fig.54 Effect of neem on carotene content of *Westiellopsis prolifica* up to 42 days of incubation.

Table 53. Effect of neem on the protein content (μg. ml^{-1} culture) of *Westiellopsis prolifica* up to 42 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation					
	7	14	21	28	35	42
Control	31.72 ± 0.08	43.33 ± 0.09	51.52 ± 0.11	59.16 ± 0.07	64.34 ± 0.09	74.37 ± 0.08
20	43.36 ± 0.09	52.17 ± 0.07	63.94 ± 0.06	71.13 ± 0.05	75.37 ± 0.011	80.28 ± 0.08
40	49.83 ± 0.11	58.36 ± 0.05	66.83 ± 0.08	72.45 ± 0.07	80.39 ± 0.06	86.34 ± 0.06
60	52.63 ± 0.08	63.33 ± 0.06	69.13 ± 0.07	79.63 ± 0.08	84.16 ± 0.06	92.27 ± 0.05
80	59.55 ± 0.06	69.73 ± 0.07	75.37 ± 0.05	83.71 ± 0.09	91.62 ± 0.05	98.33 ± 0.04
100	61.34 ± 0.09	68.28 ± 0.09	77.22 ± 0.07	88.83 ± 0.05	98.33 ± 0.04	106.21 ± 0.07
200	66.72 ± 0.08	71.31 ± 0.011	86.94 ± 0.06	92.45 ± 0.06	108.38 ± 0.05	114.76 ± 0.08
300	70.83 ± 0.12	75.87 ± 0.10	98.19 ± 0.05	106.08 ± 0.07	113.76 ± 0.10	120.82 ± 0.05
400	68.72 ± 0.11	73.45 ± 0.09	94.27 ± 0.08	101.34 ± 0.09	110.45 ± 0.06	118.49 ± 0.07

Values represent mean of three replicates ± S.D.

Table 54. Effect of neem on chlorophyll-a content (μg. ml^{-1} culture) of *Scytonema cincinnatum* on 14th day of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

Control	Concentration (ppm)							
	20	40	60	80	100	200	300	400
1.67 ± 0.05	1.23 ± 0.07	0.97 ± 0.06	0.92 ± 0.04	0.86 ± 0.05	0.72 ± 0.05	0.63 ± 0.06	0.58 ± 0.07	0.45 ± 0.08

Values represent mean of three replicates ± S.D.

Table 55. Effect of neem on carotene content (μg. ml^{-1} culture) of *Scytonema cincinnatum* on14th day of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

Control	Concentration (ppm)							
	20	40	60	80	100	200	300	400
0.147 ± 0.08	0.126 ± 0.07	0.110 ± 0.06	0.107 ± 0.06	0.092 ± 0.05	0.083 ± 0.06	0.072 ± 0.04	0.067 ± 0.06	0.51 ± 0.07

Values represent mean of three replicates ± S.D.

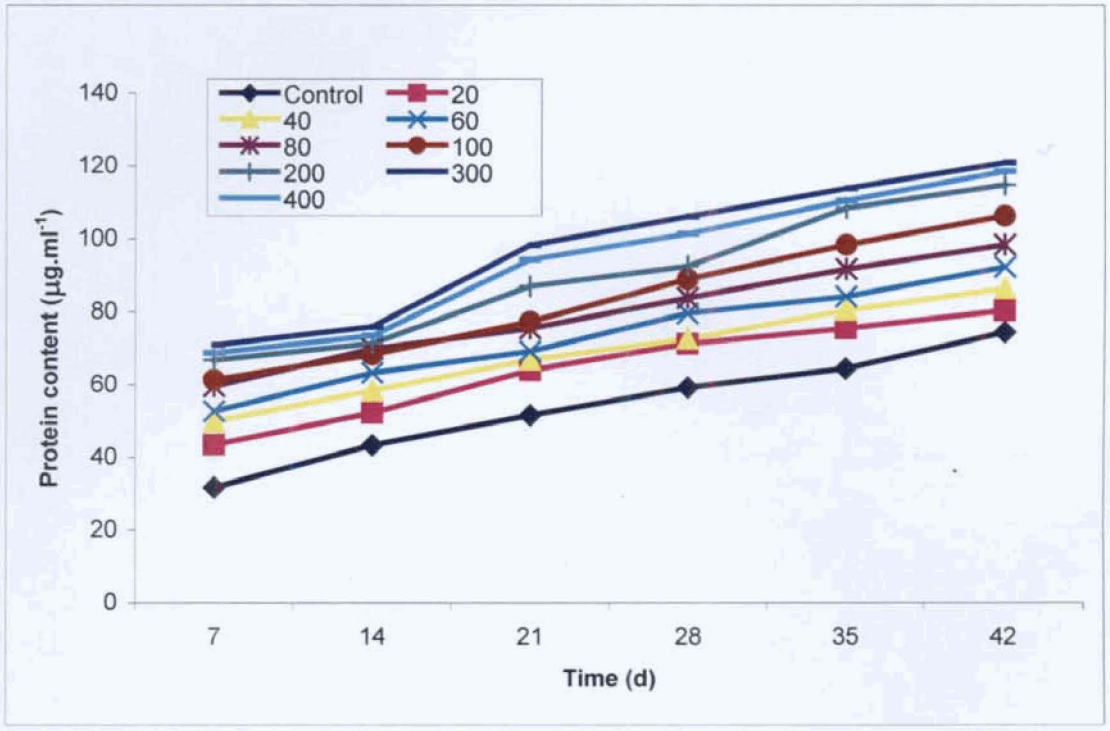


Fig.55 Effect of neem on protein content of *Westiellopsis prolifica* up to 42 days of incubation.

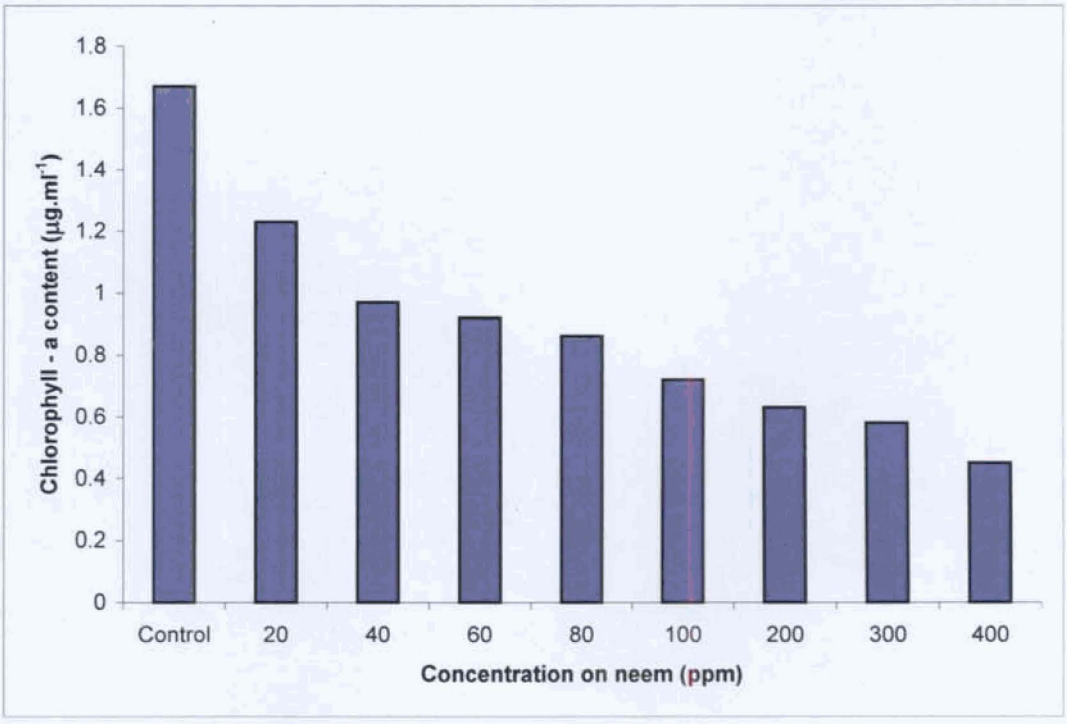


Fig.56 Effect of neem on chlorophyll-a content of *Scytonema cincinnatum* on 14th day of incubation.

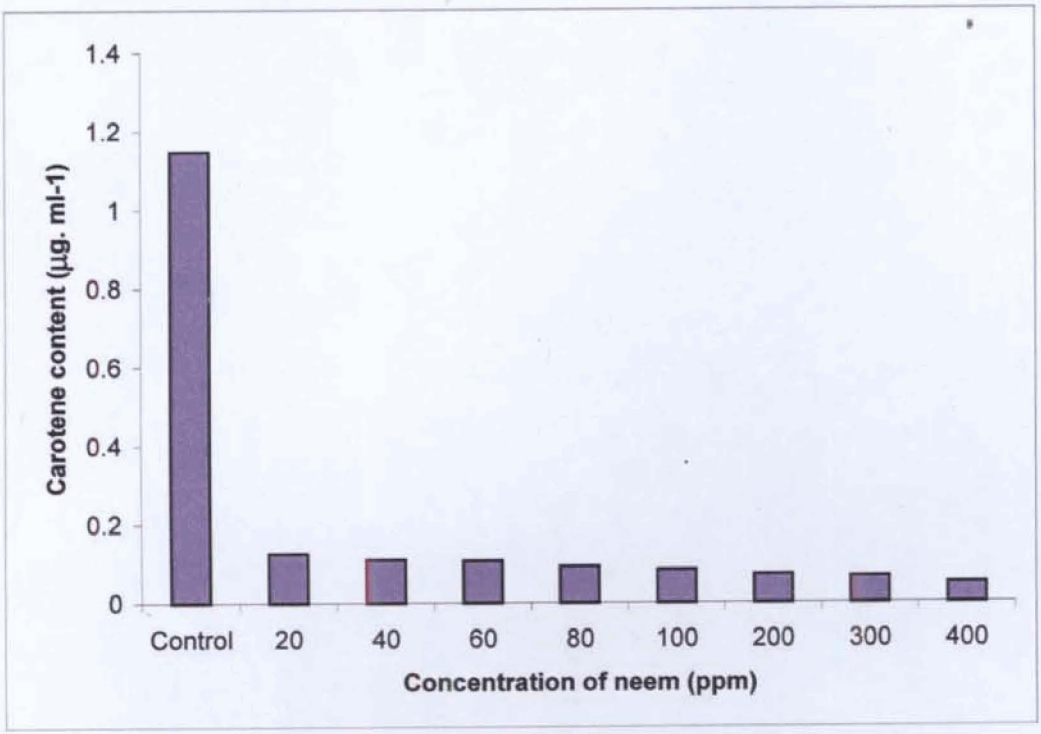


Fig.57 Effect of neem on carotene content of *Scytonema cincinnatum* on 14th day of incubation.

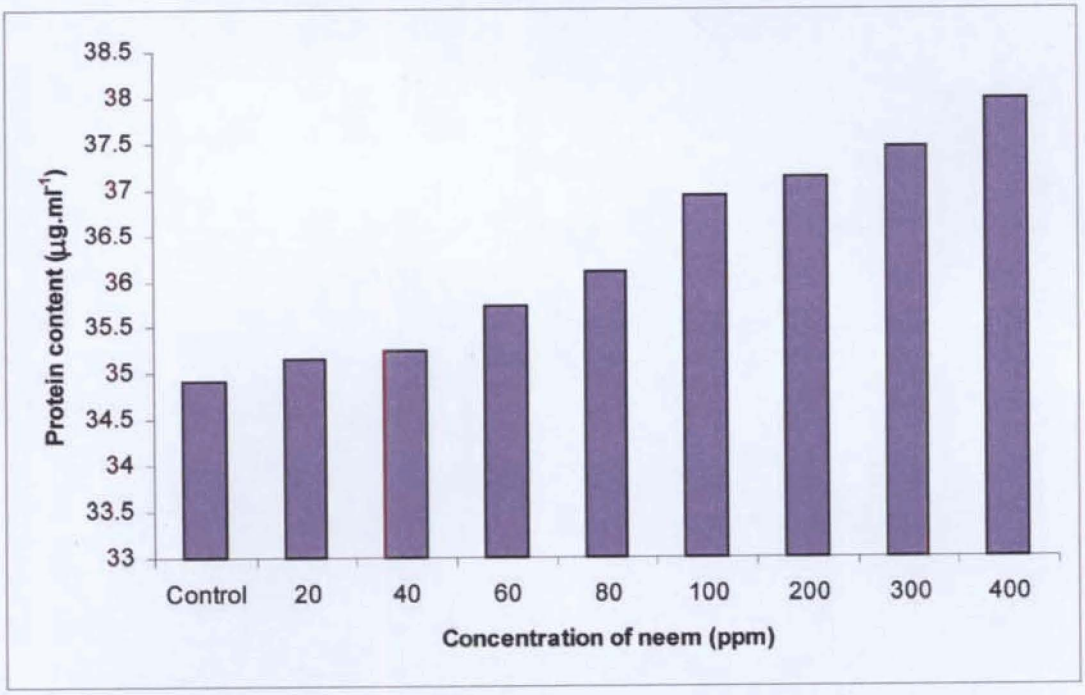


Fig.58 Effect of neem on protein content of *Scytonema cincinnatum* on 14th day of incubation.

Table 56. Effect of neem on protein content (μg. ml^{-1} culture) of *Scytonema cincinnatum* on 14th day of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

Control	Concentration (ppm)							
	20	40	60	80	100	200	300	400
34.93 ± 0.09	35.17 ± 0.06	35.24 ± 0.06	35.72 ± 0.07	36.11 ± 0.03	36.92 ± 0.08	37.12 ± 0.07	37.45 ± 0.09	37.98 ± 0.06

Values represent mean of three replicates ± S.D.

Table 57. Effect of neem on phycobiliprotein content (μg. ml^{-1} culture) of *Westiellopsis prolifica* on 42nd day of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

	Concentration (ppm)								
	Control	20	40	60	80	100	200	300	400
PC	34.62 ± 0.11	35.71 ± 0.09	35.95 ± 0.08	37.41 ± 0.09	38.16 ± 0.07	38.95 ± 0.09	44.18 ± 0.08	48.47 ± 0.011	49.03 ± 0.10
APC	36.34 ± 0.10	36.97 ± 0.10	37.46 ± 0.09	38.87 ± 0.11	39.45 ± 0.10	40.14 ± 0.07	46.27 ± 0.05	52.14 ± 0.08	53.97 ± 0.09
PE	3.26 ± 0.12	3.84 ± 0.11	4.95 ± 0.10	5.04 ± 0.12	5.27 ± 0.11	6.52 ± 0.08	6.97 ± 0.07	7.21 ± 0.09	7.83 ± 0.07

Values represent mean of three replicates ± S.D.

Table 58. Effect of neem on phycobiliprotein content (μg. ml^{-1} culture) of *Scytonema cincinnatum* on 14th day of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

	Concentration (ppm)								
	Control	20	40	60	80	100	200	300	400
PC	20.13 ± 0.10	20.34 ± 0.09	20.82 ± 0.08	21.06 ± 0.11	21.37 ± 0.12	21.72 ± 0.11	22.03 ± 0.07	22.45 ± 0.12	22.91 ± 0.07
APC	18.21 ± 0.12	18.36 ± 0.11	18.57 ± 0.10	18.98 ± 0.12	19.26 ± 0.09	19.83 ± 0.07	19.94 ± 0.09	20.11 ± 0.11	20.69 ± 0.09
PE	1.72± 0.11	1.75 ± 0.07	1.92 ± 0.11	2.05 ± 0.06	2.16 ± 0.08	2.57 ± 0.09	2.71 ± 0.10	2.87 ± 0.09	2.95 ± 0.10

Values represent mean of three replicates ± S.D.

Table 59. Excretion of ammonia (n moles ml^{-1}) at different concentrations of neem on 14th day of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

	Concentration (ppm)								
Cyanobacteria	Control	20	40	60	80	100	200	300	400
Westiellopsis prolifica	35.12 ± 0.06	37.46 ± 0.09	39.37 ± 0.11	44.01 ± 0.05	49.56 ± 0.04	51.03 ± 0.09	53.74 ± 0.08	54.07 ± 0.08	55 ± 0.09
Scytonema cincinnatum	29.34 ± 0.08	25.41 ± 0.07	22.62 ± 0.10	21.31 ± 0.09	19.42 ± 0.06	18.64 ± 0.06	18.11 ± 0.07	17.36 ± 0.06	15.01 ± 0.05

Values represent mean of three replicates ± S.D.

Table 60. Heterocyst abundance at different concentrations of neem after 42 days of incubation. Cultures were incubated at 25 ± 1°C with 12h light/dark cycle

	Concentration (ppm)								
Cyanobacteria	Control	20	40	60	80	100	200	300	400
Westiellopsis prolifica	11.94 ± 0.06	12.1 ± 0.10	12.4 ± 0.05	12.9 ± 0.03	13.1 ± 0.05	13.5 ± 0.07	13.7 ± 0.05	13.9 ± 0.06	13.9 ± 0.05
Scytonema cincinnatum	8.5 ± 0.09	8.1 ± 0.11	7.6 ± 0.07	6.2 ± 0.04	5.9 ± 0.05	5.1 ± 0.06	4.8 ± 0.05	4.2 ± 0.03	3.8 ± 0.06

Values represent mean of three replicates ± S.D.

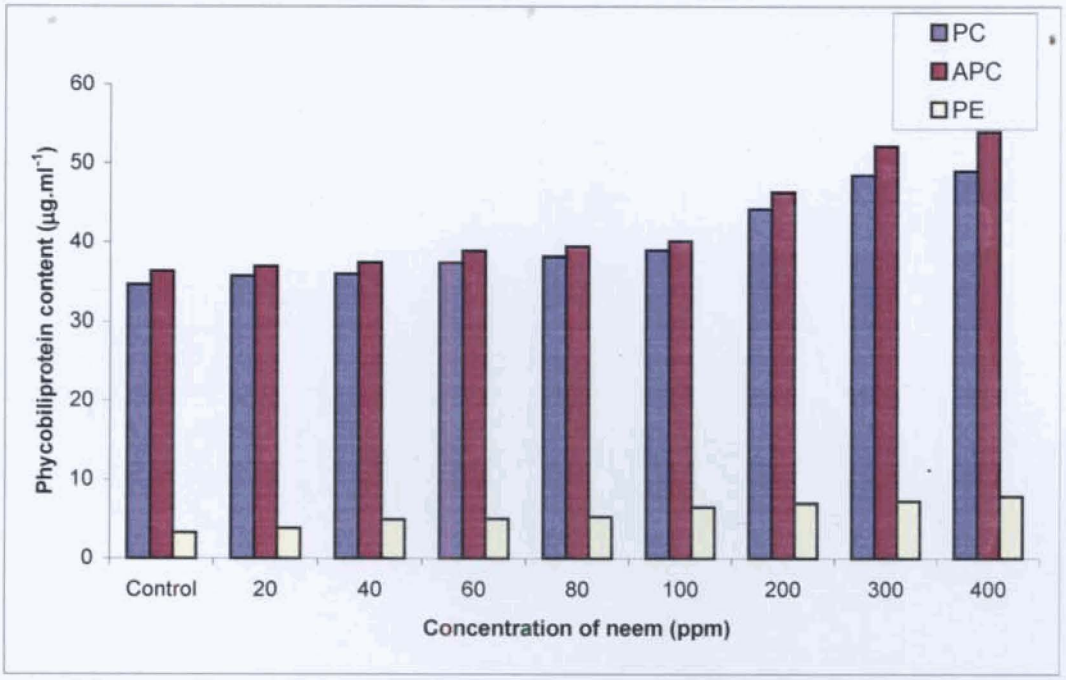


Fig.59 Effect of neem on phycobiliprotein content of *Westiellopsis prolifica* on 42nd day of incubation.

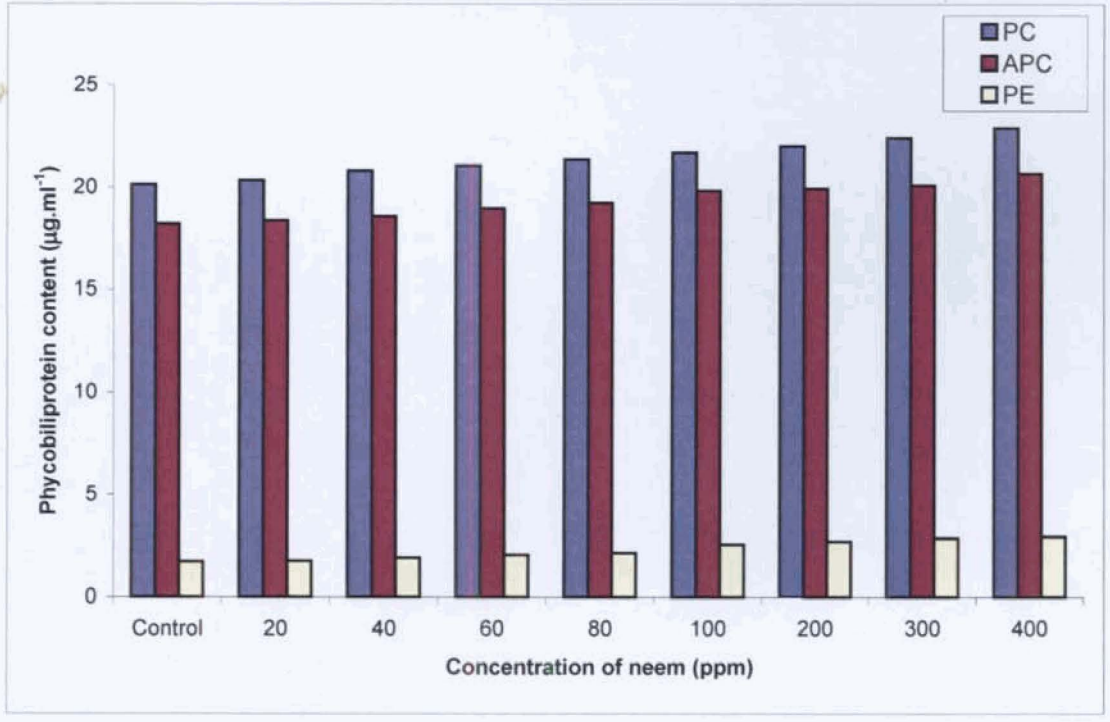


Fig.60 Effect of neem on phycobiliprotein content of *Scytonema cincinnatum* on 14th day of incubation.

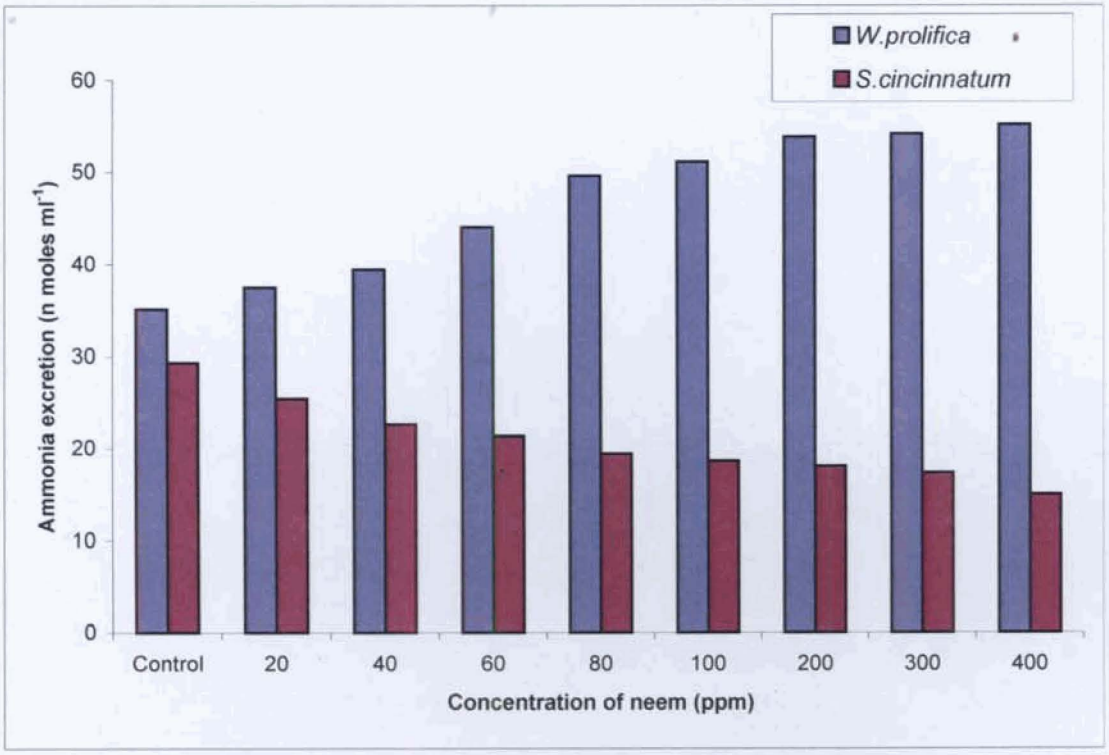


Fig.61 Effect of neem on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 14th day of incubation.

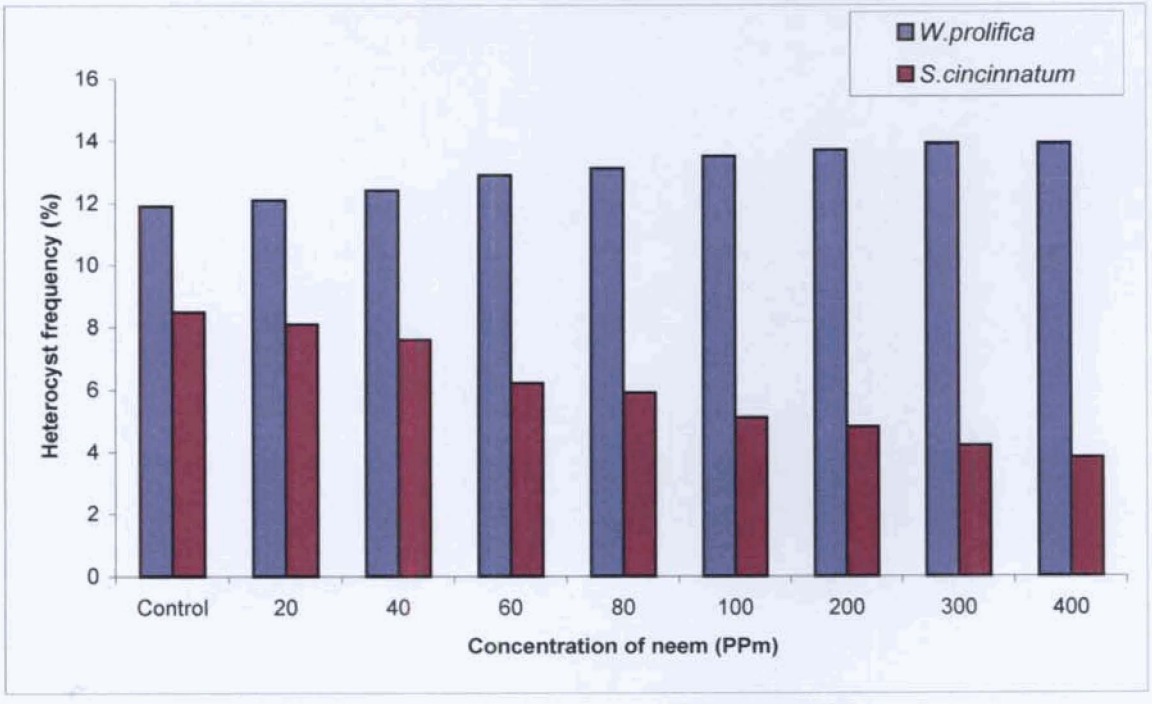


Fig.62 Effect of neem on heterocyst abundance of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 42nd day of incubation.

Morphological studies on *Westiellopsis prolifica* showed that at 200, 300 and 400 ppm concentrations of neem, the vegetative cells and heterocyst are larger in size and branching frequency was also frequent. After 35 days of growth, akinetes and spores were produced. But in *Scytonema cincinnatum*, the cells get disintegrated after two weeks of growth in higher concentrations of neem solution.

4.6.2. Discussion

Cyanobacterial biofertilizers were widely used for inoculation in rice fields for the supply of nitrogen to rice crop. Excretion of organic acids by cyanobacteria have shown to improve the physical condition of the soil and phosphorous availability in rice fields. The nitrogen demand of rice when supplemented with nitrogen supply from incorporated organic residues would increase nitrogen use efficiency of rice and reduce soil nitrogen losses (Kannaiyan, 1980).

The insecticide neem cake is generally used as organic fertilizer for rice crop and it is known to inhibit the nitrification process. Neem cake contains the alkaloids such as azadiractin, nimbin and nimbidin which are responsible for affecting the activity of nitrifying bacteria such as *Nitrosomonas* and *Nitrobactor* (Sukumar *et al.*, 1988). Manickam *et al.* (1976) have demonstrated that the application of neem oil has a positive nitrification inhibitor effect when mixed along with fertilizer nitrogen for rice crop. Nitrification inhibitors are known to regulate nitrogen supply and thereby increasing efficiency of fertilizer nitrogen uptake which directly

influences the dry matter production and grain yield of rice (De Datta, 1986). There are reports that the application of nitrification inhibitors alongwith urea in lowland rice could inhibit nitrification (De Datta *et al.*, 1983) and suppress the transformation of ammoniacal nitrogen and thereby accumulate more ammoniacal nitrogen for effective utilization in rice crop (Shankar *et al.*, 1976).

The present investigation suggests that neem cake has got a positive role in augmenting the pigment synthesis, protein content and ammonia excretion which in turn might influence the biomass productivity in *Westiellopsis prolifica* while it has got a negative role in *Scytonema cincinnatum.* The favourable effect of neem cake on the growth and nitrogen fixation in *Westiellopsis prolifica* could be due to many reasons. It is known to have properties of being a nitrification inhibitor which has got inhibitory action on nitriters (Khandelwal *et al.*, 1977).

There are reports that neem cake had stimulating effects on the growth and multiplication of free living cyanobacteria (Aziz *et al.*, 1981; Kannaiyan *et al.*, 1983; Kanniyan and Sundaravarathan, 1998; Reddy and Prasad, 1975). Subbaiah and Kothandaraman (1980) reported that neem cake acts as a nitrification inhibitor and thus reduces loss of applied nitrogen. Coating of prilled urea with neem cake or neem seed extract was considered to be a promising practice for enhancing nitrogen use efficiency by reducing the nitrogen loss and regulating the nitrogen availability in rice (Shankar *et al.*, 1976) and also increases the uptake of nitrogen, phosphorous and

potassium (Harishankar and Rathi, 1976), and yield of rice (Kannaiyan *et al.*, 1983). There are also reports that the application of neem cake reduces the pest incidence in *Azolla pinnata* (Kannaiyan *et al.*, 1983; Nandabalan and Kannaiyan, 1984). Thus it was apparently clear from the study that the nitrification inhibitor neem cake has positive influence on the growth of *Westiellopsis prolifica* than *Scytonema cinncinnatum.* The production of ammonia at higher concentrations of neem in *Scytonema cincinnatum* may be due to the liberation of ammonia after the death of the cells. Since the cost of neem cake is comparatively very low, it can be exploited for biomass production of *Westiellopsis prolifica* which can in turn utilized for rice cultivation in low land paddy fields.

4.7. EFFECT OF HERBICIDE 2,4-D ON THE GROWTH AND NITROGEN FIXATION OF *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM*

4.7.1. Results

The effect of different concentration of 2,4-D on growth, pigmentation, protein content and ammonia excretion of *Westiellopsis prolifica* and *Scytonema cincinnatum* were examined. The extend of sensitivity was slightly different in both the test organisms. Results obtained indicated that 2,4-D stimulated the growth upto 5 ppm in *Westiellopsis prolifica* and up to 1 ppm in *Scytonema cincinnatum* respectively over the control. Relatively higher concentrations (above 10 ppm) proved lethel for both *Westiellopsis prolifica* and *Scytonema cincinnatum*, eventhough it can tolerate up to 100

ppm (Table 61, 62 & Fig. 63, 64). In *Scytonema cincinnatum* there was only a marginal decrease in biomass content at 5 ppm 2,4-D. Thus *Westiellopsis prolifica* showed better growth than *Scytonema cincinnatum.* The chlorophyll, carotene, proteins and phycobilin pigments decreased with increasing concentration of herbicide and showed a trend similar to that of biomass production (Table 63-74 & Fig. 65-76). A decrease in ammonia production was observed with increasing concentration of herbicide. The release of ammonia was more in *Westiellopsis prolifica* than in *Scytonema cincinnatum* (Table 75 & Fig. 77).

4.7.2. Discussion

In the present day agriculture, weed control using herbicides is a common practice. Use of many high yielding varieties of paddy has necessitated routine application of variety of agrochemicals including insecticides and herbicides. A considerable amount of these herbicides get access to terrestrial ecosystems and affect the non target organisms. Many of them are potential biofertilizers and help in improving crop yields (Likhitkar and Tarar, 1995). Herbicides impose chemical stress which influences the biological activity of cyanobacteria (Roychoudhury and Kaushik, 1986; Goyal *et al.*, 1991).

The increasing use of herbicides to rice fields particularly 2,4-dichlorophenoxy acetic acid (2,4-D), a synthetic growth hormone analogue is likely to affect the growth of cyanobacteria. In the present investigation, 2,4-D has been observed to be toxic to *Westiellopsis prolifica* and

Scytonema cincinnatum above 10 ppm. The results indicated that the level of herbicide used strongly affected the cyanobacterial growth and biochemical metabolites. Both the cyanobacteria exhibited a differential degree of sensitivity to 2,4-D. The possible reason for such behaviour of the cyanobacterial species may be the differential permeability of this herbicide across the cell membrane. On analysing the concentration dependent influence of 2,4-D on cyanobacteria, it was seen that up to 5 ppm, the herbicide was stimulatory for *Westiellopsis prolifica*, however, more than 5 ppm proved to be inhibitory to biomass production, chlorophyll synthesis and nitrogen fixation. The effect of herbicide on pigment synthesis in turn reduces the rate of photosynthesis, cell division and finally the growth rate. In *Scytonema cincinnatum* 1 ppm 2,4-D is stimulatory while 5 ppm shows an inhibitory effect. However, a complete inhibition was not observed even up to 50 ppm during the present investigation and reduced growth could be seen even at 100 ppm, suggesting the variations in tolerance level at generic level. There are reports that *Westiellopsis prolifica* can tolerate up to 1500 $\mu g\ ml^{-1}$ 2,4-D (Nanda and Padhi, 1992). A stimulatory effect on the cyanobacteria at low concentration of herbicide was attributed to the direct effect exerted by utilization of either chemical it self or its degradory products (Goyal *et al.*, 1991).

Table 61. Effect of 2,4-D on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* up to 28 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	0.25 ± 0.17	0.31 ± 0.15	0.39 ± 0.14	0.42 ± 0.16	0.54 ± 0.14
0.5	0.26 ± 0.11	0.33 ± 0.11	0.41 ± 0.13	0.46 ± 0.11	0.58 ± 0.12
1	0.27 ± 0.10	0.35 ± 0.17	0.45 ± 0.12	0.50 ± 0.09	0.62 ± 0.11
5	0.27 ± 0.15	0.38 ± 0.16	0.47 ± 0.11	0.51 ± 0.10	0.64 ± 0.12
10	0.22 ± 0.14	0.24 ± 0.18	0.28 ± 0.15	0.29 ± 0.15	0.25 ± 0.15
50	0.18 ± 0.10	0.20 ± 0.14	0.22 ± 0.10	0.21 ± 0.16	0.17 ± 0.13
100	0.16 ± 0.11	0.15 ± 0.11	0.14 ± 0.16	0.12 ± 0.14	0.10 ± 0.15

Values represent mean of three replicates ± S.D.

Table 62. Effect of 2,4-D on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	0.24 ± 0.19	0.26 ± 0.10	0.30 ± 0.13	0.33 ± 0.11	0.37 ± 0.10
0.5	0.02 ± 0.15	0.26 ± 0.10	0.34 ± 0.12	0.38 ± 0.10	0.40 ± 0.11
1	0.28 ± 0.15	0.33 ± 0.11	0.39 ± 0.11	0.42 ± 0.10	0.45 ± 0.11
5	0.22 ± 0.10	0.31 ± 0.12	0.35 ± 0.15	0.36 ± 0.12	0.39 ± 0.09
10	0.19 ± 0.12	0.21 ± 0.15	0.22 ± 0.15	0.20 ± 0.18	0.18 ± 0.12
50	0.15 ± 0.12	0.14 ± 0.14	0.13 ± 0.12	0.11 ± 0.15	0.10 ± 0.13
100	0.13 ± 0.10	0.13 ± 0.14	0.14 ± 0.10	0.13 ± 0.16	0.12 ± 0.12

Values represent mean of three replicates ± S.D.

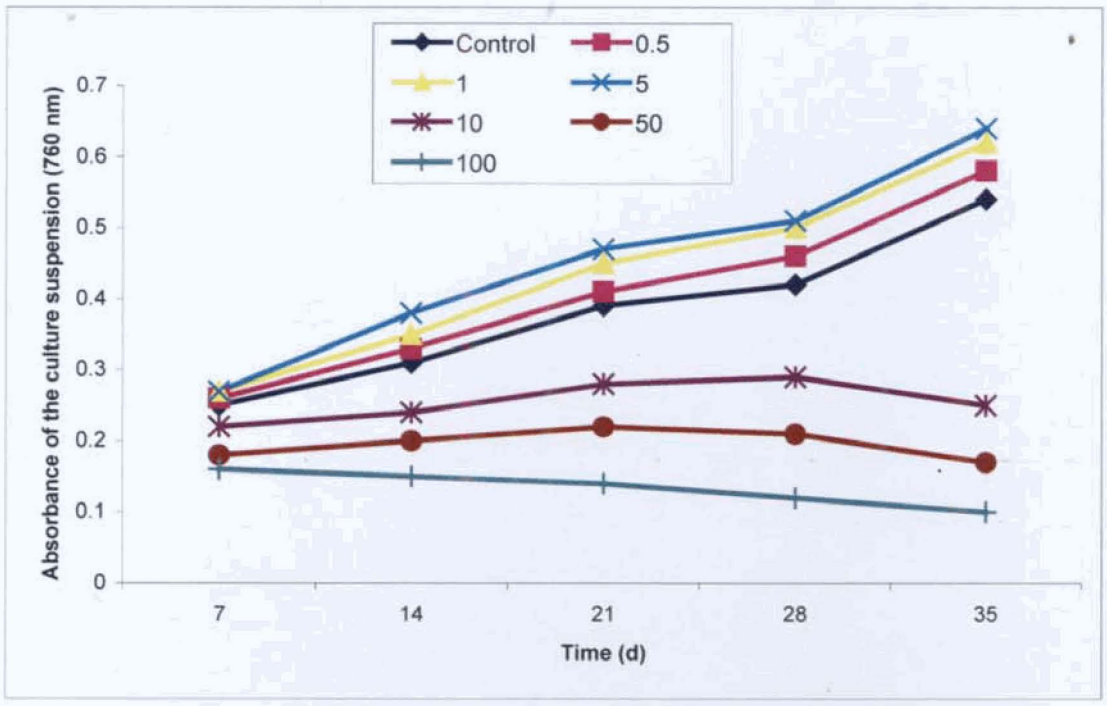


Fig.63 Effect of 2,4-D on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* up to 42 days of incubation.

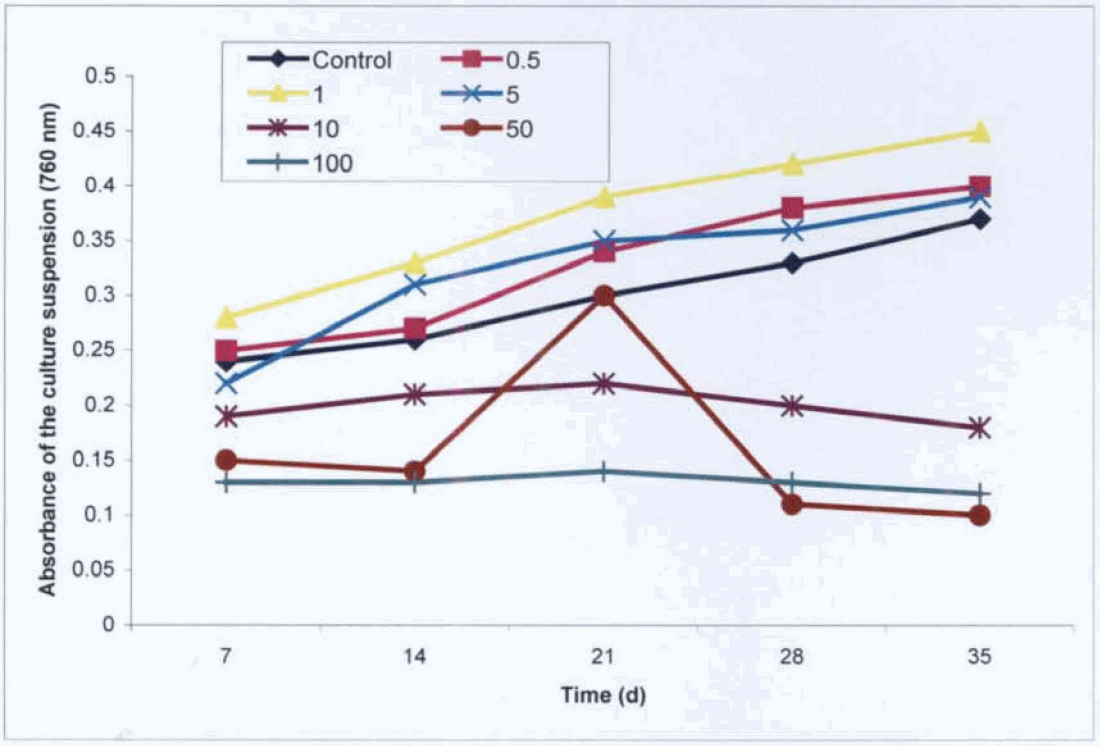


Fig.64 Effect of 2,4-D on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 35 days of incubation.

Table 63. Effect of 2,4-D on chlorophyll-a content (μg. ml^{-1} culture) of *Westielliopsis prolifica* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	0.98 ± 0.11	1.69 ± 0.04	2.64 ± 0.05	3.04 ± 0.09	3.45 ± 0.04
0.5	1.08 ± 0.05	1.84 ± 0.03	2.71 ± 0.06	3.15 ± 0.05	3.53 ± 0.06
1	1.15 ± 0.04	1.95 ± 0.04	2.79 ± 0.06	3.21 ± 0.04	3.62 ± 0.08
5	1.27 ± 0.09	2.01 ± 0.04	2.85 ± 0.07	3.32 ± 0.07	3.66 ± 0.04
10	0.95 ± 0.10	1.13 ± 0.05	1.26 ± 0.03	1.29 ± 0.07	1.21 ± 0.03
50	0.83 ± 0.06	0.89 ± 0.03	0.93 ± 0.03	0.95 ± 0.06	0.94 ± 0.04
100	0.72 ± 0.05	0.75 ± 0.02	0.78 ± 0.05	0.76 ± 0.05	0.61 ± 0.10

Values represent mean of three replicates ± S.D.

Table 64. Effect of 2,4-D on chlorophyll-a content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	0.83 ± 0.06	1.03 ± 0.08	2.46 ± 0.10	2.94 ± 0.09	3.21 ± 0.06
0.5	0.89 ± 0.07	1.14 ± 0.09	2.49 ± 0.11	2.98 ± 0.06	3.32 ± 0.08
1	0.98 ± 0.09	1.21 ± 0.08	2.52 ± 0.09	3.12 ± 0.05	3.43 ± 0.05
5	0.84 ± 0.11	1.16 ± 0.07	2.41 ± 0.06	2.64 ± 0.09	2.94 ± 0.08
10	0.76 ± 0.10	0.79 ± 0.-6	0.83 ± 0.09	0.81 ± 0.06	0.72 ± 0.06
50	0.64 ± 0.12	0.66 ± 0.09	0.65 ± 0.05	0.63 ± 0.09	0.61 ± 0.09
100	0.43 ± 0.09	0.45± 0.10	0.41 ± 0.04	0.42 ± 0.05	0.38 ± 0.05

Values represent mean of three replicates ± S.D.

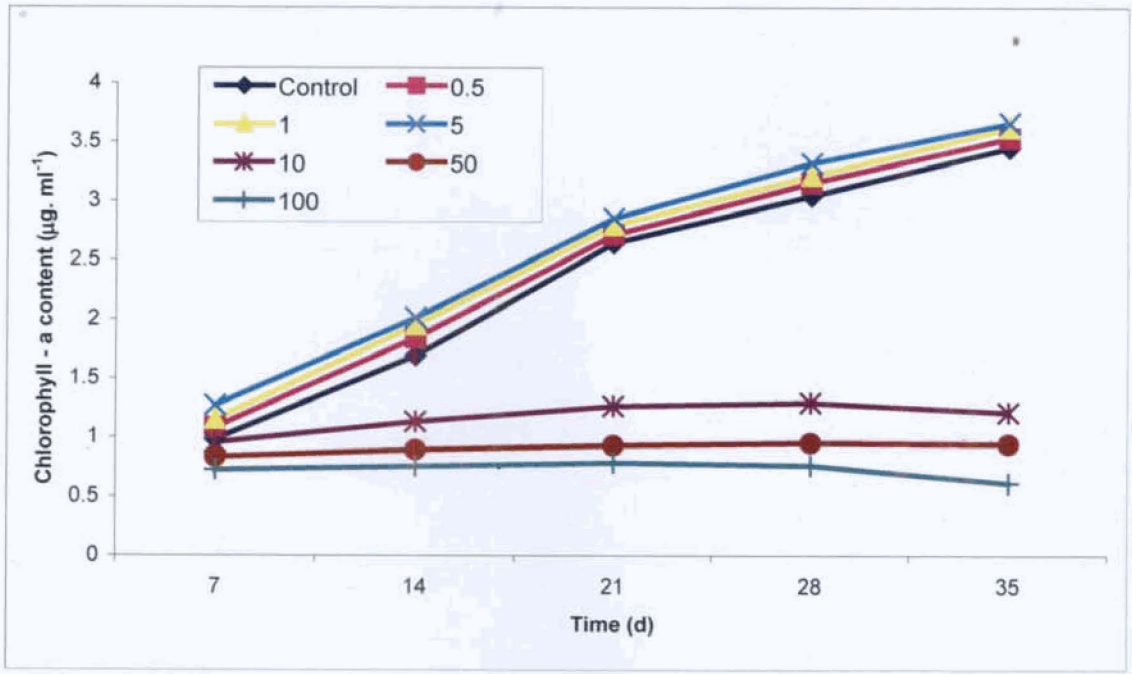


Fig.65 Effect of 2,4-D on chlorophyll-a content of *Westiellopsis prolifica* up to 35 days of incubation.

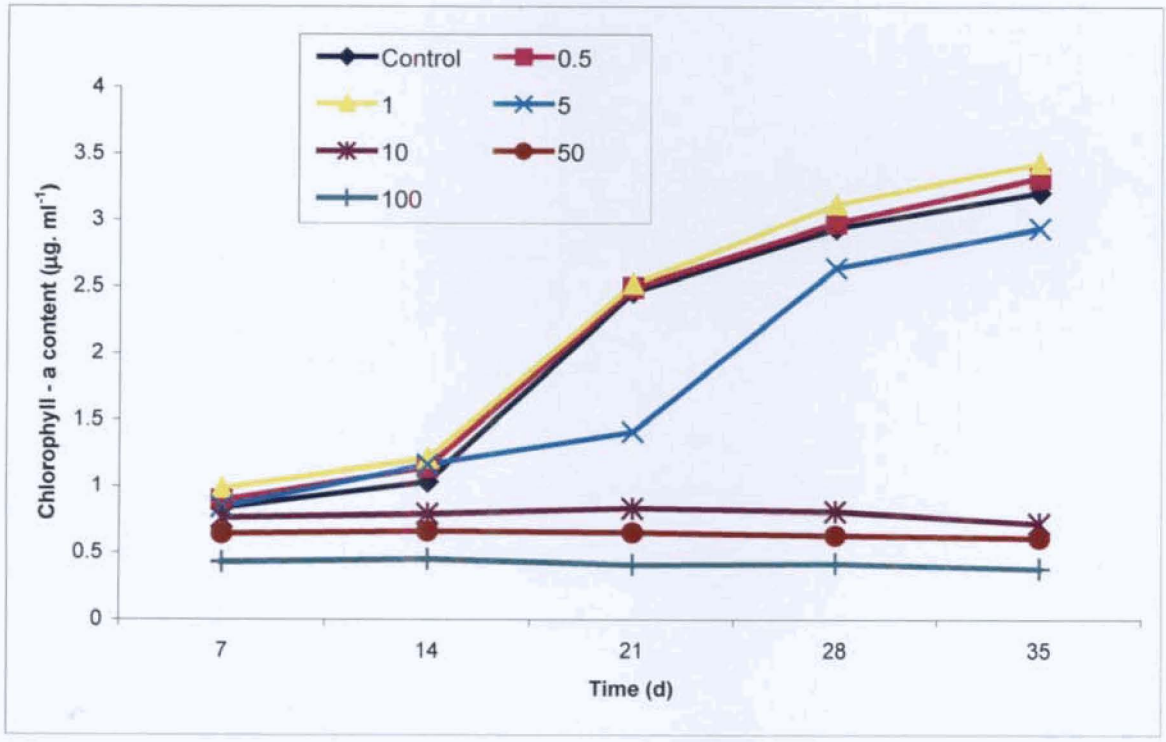


Fig.66 Effect of 2,4-D on chlorophyll-a content of *Scytonema cincinnatum* up to 35 days of incubation.

Table 65. Effect of 2,4-D on carotene content (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 35 days of incubation at 25 ± 1°C under with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	0.219 ± 0.06	0.465 ± 0.04	0.684 ± 0.06	0.712 ± 0.04	0.741 ± 0.05
0.5	0.220 ± 0.01	0.468 ± 0.06	0.689 ± 0.02	0.733 ± 0.05	0.744 ± 0.05
1	0.224 ± 0.06	0.472 ± 0.03	0.694 ± 0.02	0.742 ± 0.06	0.748 ± 0.02
5	0.226 ± 0.05	0.479 ± 0.02	0.699 ± 0.03	0.749 ± 0.03	0.751 ± 0.03
10	0.215 ± 0.04	0.219 ± 0.03	0.234 ± 0.02	0.221 ± 0.03	0.219 ± 0.03
50	0.211 ± 0.03	0.216 ± 0.04	0.218 ± 0.05	0.210 ± 0.02	0.208 ± 0.02
100	0.208 ± 0.02	0.210 ± 0.05	0.211 ± 0.02	0.201 ± 0.03	0.119 ± 0.04

Values represent mean of three replicates ± S.D.

Table 66. Effect of 2,4-D on carotene content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	0.143 ± 0.06	0.154 ± 0.11	0.196 ± 0.09	0.291 ± 0.08	0.312 ± 0.06
0.5	0.144 ± 0.05	0.156 ± 0.05	0.199 ± 0.04	0.294 ± 0.09	0.296 ± 0.09
1	0.146 ± 0.08	0.159 ± 0.09	0.205 ± 0.06	0.298 ± 0.10	0.301 ± 0.05
5	0.141 ± 0.08	0.142 ± 0.06	0.149 ± 0.06	0.150 ± 0.10	0.138 ± 0.09
10	0.117 ± 0.07	0.119 ± 0.05	0.121± 0.04	0.118 ± 0.11	0.111 ± 0.06
50	0.115 ± 0.09	0.106 ± 0.10	0.108 ± 0.09	0.113 ± 0.10	0.109 ± 0.04
100	0.103 ± 0.10	0.104 ± 0.11	0.105 ± 0.09	0.102 ± 0.10	0.101 ± 0.09

Values represent mean of three replicates ± S.D.

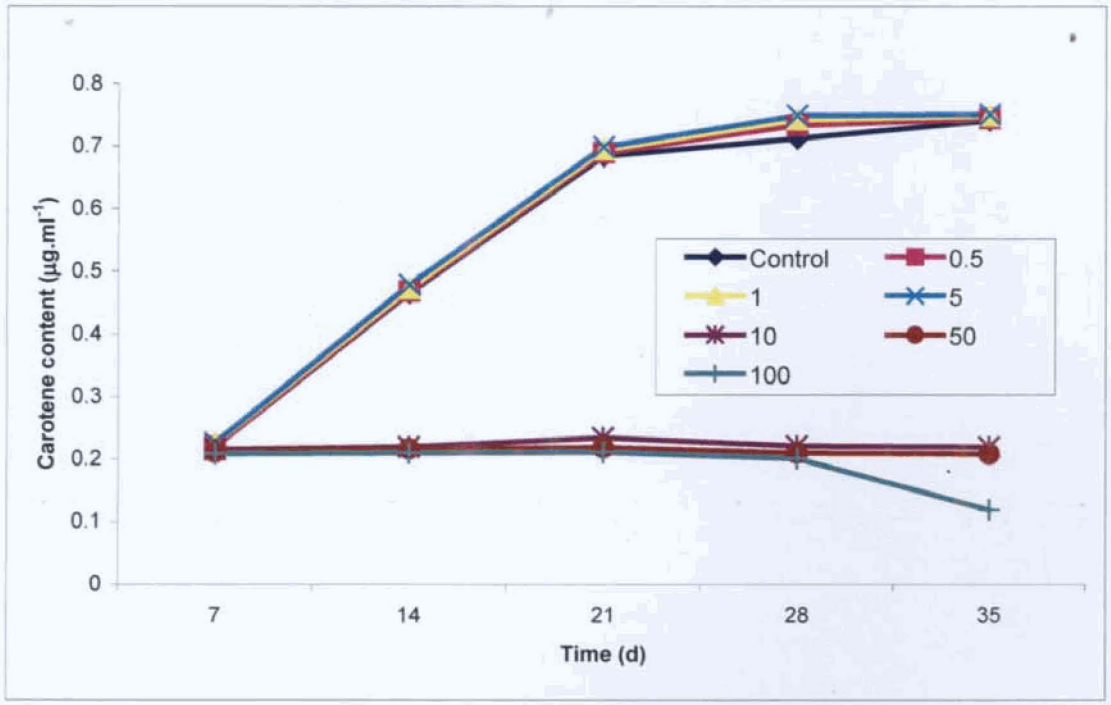


Fig.67 Effect of 2,4-D on carotene content of *Westiellopsis prolifica* up to 35 days of incubation.

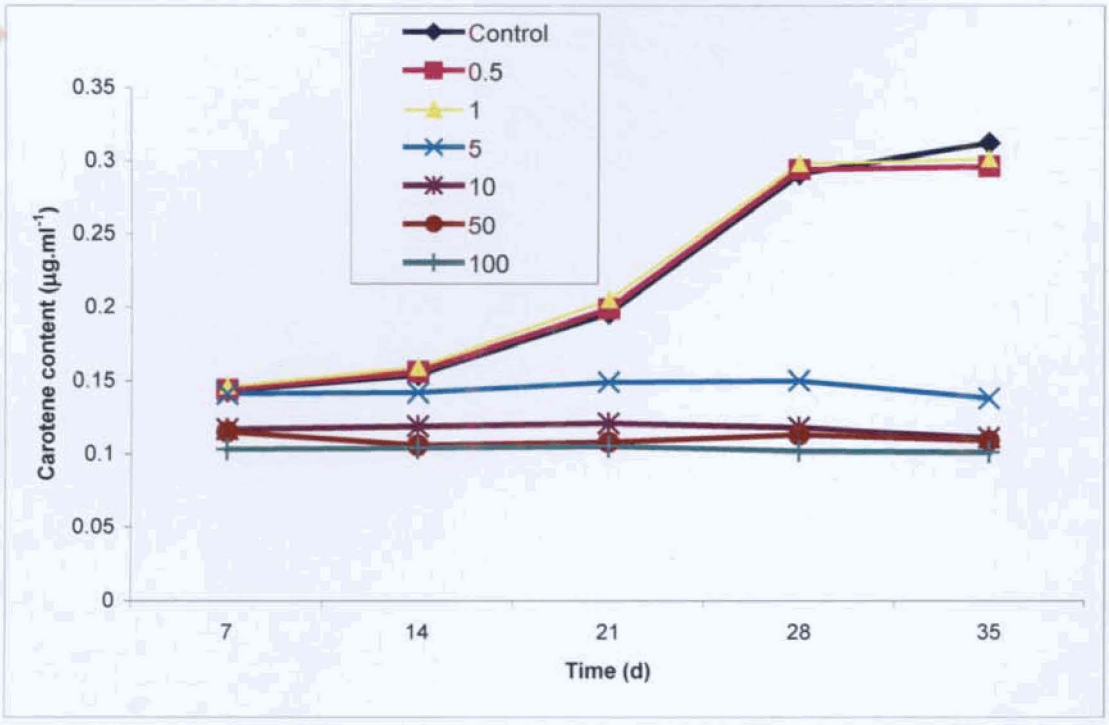


Fig.68 Effect of 2,4-D on carotene content of *Scytonema cincinnatum* up to 35 days of incubation.

Table 67. Effect of 2,4-D on protein content (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	25.26 ± 0.21	32.47 ± 0.24	45.72 ± 0.19	51.17 ± 0.14	55.43 ± 0.15
0.5	25.72 ± 0.25	33.14 ± 0.19	46.11 ± 0.18	51.74 ± 0.19	55.84 ± 0.19
1	26.34 ± 0.19	33.75 ± 0.16	46.72 ± 0.21	52.21 ± 0.16	55.91 ± 0.21
5	27.11 ± 0.22	34.08 ± 0.15	46.93 ± 0.26	52.74 ± 0.22	55.98 ± 0.22
10	21.19 ± 0.26	25.42 ± 0.21	27.18 ± 0.19	29.17 ± 0.19	25.34 ± 0.19
50	18.26 ± 0.15	18.71 ± 0.15	19.23 ± 0.15	19.84 ± 0.16	19.11 ± 0.18
100	10.11 ± 0.23	10.26 ± 0.16	10.25 ± 0.18	10.21 ± 0.16	10.19 ± 0.16

Values represent mean of three replicates ± S.D.

Table 68. Effect of 2,4-D on protein content (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	21.14 ± 0.18	29.26 ± 0.15	33.42 ± 0.14	41.72 ± 0.14	46.14 ± 0.11
0.5	21.45 ± 0.11	29.81 ± 0.10	34.17 ± 0.11	41.83 ± 0.12	46.27 ± 0.15
1	21.49 ± 0.10	29.94 ± 0.11	34.28 ± 0.11	41.92 ± 0.13	46.73 ± 0.12
5	20.11 ± 0.19	20.45 ± 0.10	21.16 ± 0.19	21.74 ± 0.12	21.78 ± 0.13
10	18.26 ± 0.11	18.74 ± 0.17	18.96 ± 0.15	17.16 ± 0.12	16.21 ± 0.10
50	15.34 ± 0.15	15.39 ± 0.15	15.41 ± 0.13	14.21 ± 0.12	14.02 ± 0.11
100	9.11 ± 0.14	9.23 ± 0.15	9.01 ± 0.12	8.47 ± 0.11	8.21 ± 0.10

Values represent mean of three replicates ± S.D.

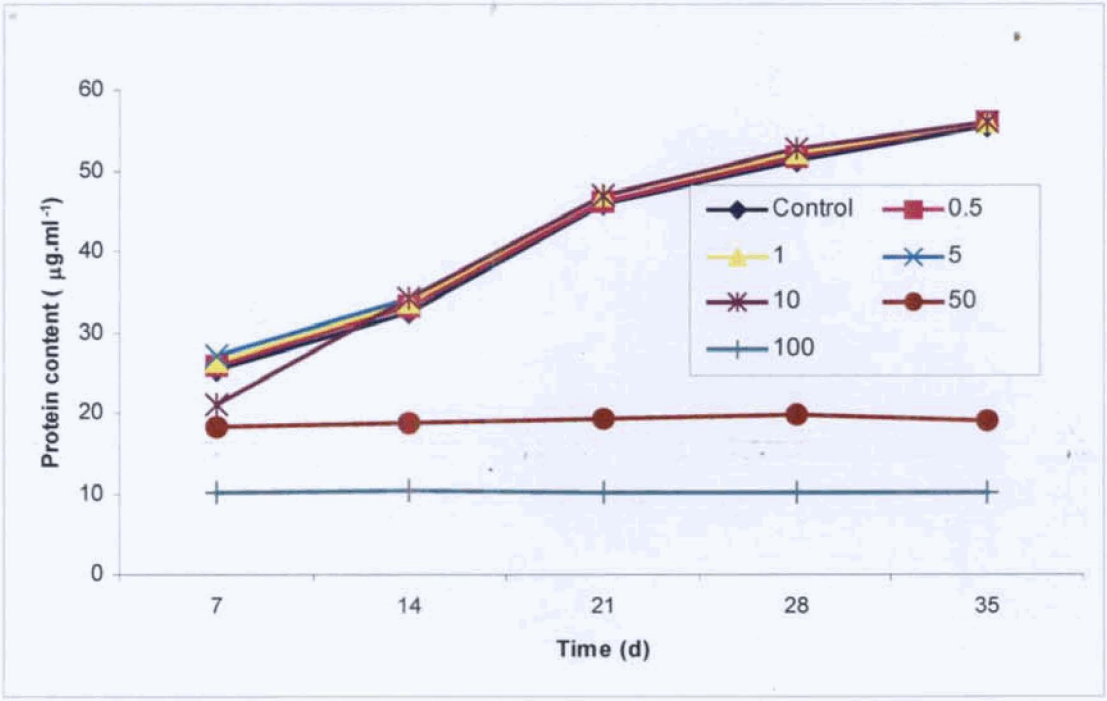


Fig.69 Effect of 2,4-D on protein content of *Westiellopsis prolifica* up to 35 days of incubation.

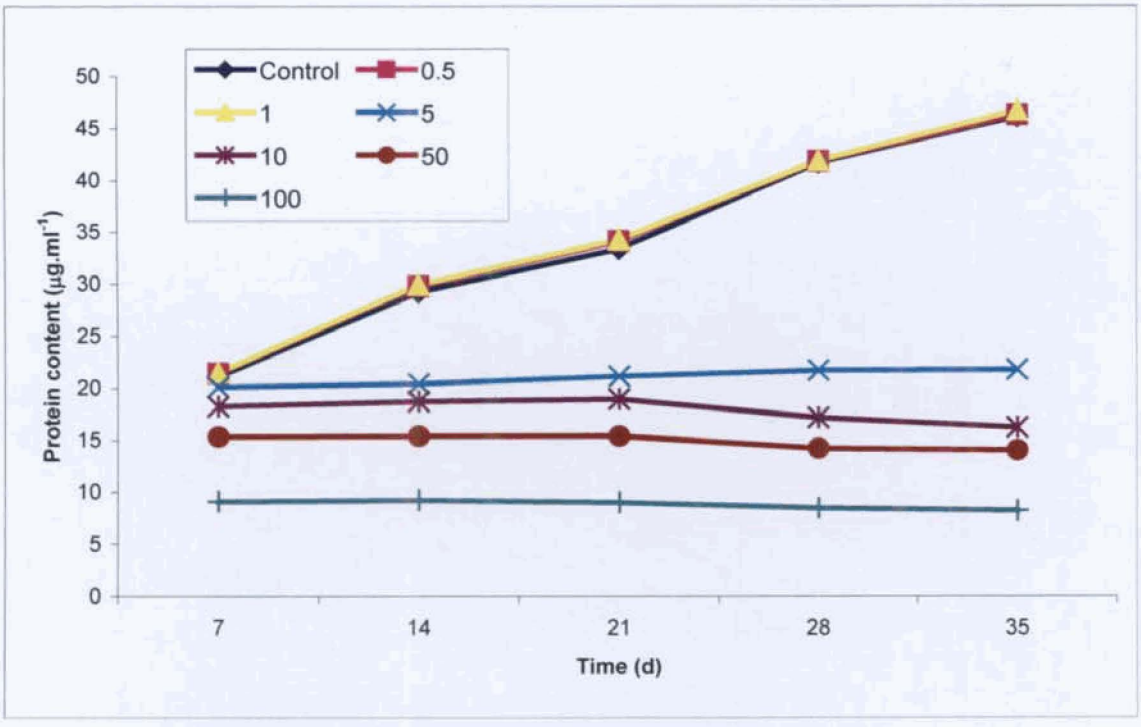


Fig.70 Effect of 2,4-D on protein content of *Scytonema cincinnatum* up to 35 days of incubation

Table 69. Effect of 2,4-D on phycobilin content c-phycocyanin (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	12.11 ± 0.07	14.17 ± 0.05	18.11 ± 0.06	21.34 ± 0.05	24.17 ± 0.09
0.5	12.13 ± 0.05	14.28 ± 0.02	18.21 ± 0.04	21.79 ± 0.09	24.29 ± 0.06
1	12.19 ± 0.06	14.62 ± 0.01	18.39 ± 0.07	22.63 ± 0.01	24.36 ± 0.02
5	12.31 ± 0.05	14.79 ± 0.03	18.75 ± 0.05	22.74 ± 0.05	24.99 ± 0.05
10	11.06 ± 0.07	11.27 ± 0.09	11.31 ± 0.04	11.14 ± 0.03	11.28 ± 0.03
50	9.98 ± 0.08	9.48 ± 0.04	9.36 ± 0.06	9.12 ± 0.06	19.03 ± 0.05
100	3.31 ± 0.06	3.22 ± 0.06	3.10 ± 0.07	3.94 ± 0.05	3.81 ± 0.02

Values represent mean of three replicates ± S.D.

Table 70. Effect of 2,4-D on phycobilin content allophycocyanin (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	15.11 ± 0.05	19.21 ± 0.05	24.34 ± 0.04	27.27 ± 0.03	30.14 ± 0.06
0.5	15.26 ± 0.04	19.28 ± 0.08	24.36 ± 0.09	27.34 ± 0.08	30.56 ± 0.05
1	15.67 ± 0.07	19.92 ± 0.02	24.48 ± 0.05	27.98 ± 0.05	30.92 ± 0.04
5	12.31 ± 0.10	12.34 ± 0.03	12.39 ± 0.02	12.41 ± 0.05	12.14 ± 0.07
10	11.02 ± 0.09	10.61 ± 0.05	10.11 ± 0.01	9.83 ± 0.03	9.71 ± 0.02
50	7.26 ± 0.07	7.14 ± 0.06	7.17 ± 0.06	7.03 ± 0.04	6.04 ± 0.02
100	2.44 ± 0.08	2.33 ± 0.07	2.24 ± 0.02	2.15 ± 0.05	2.07 ± 0.04

Values represent mean of three replicates ± S.D.

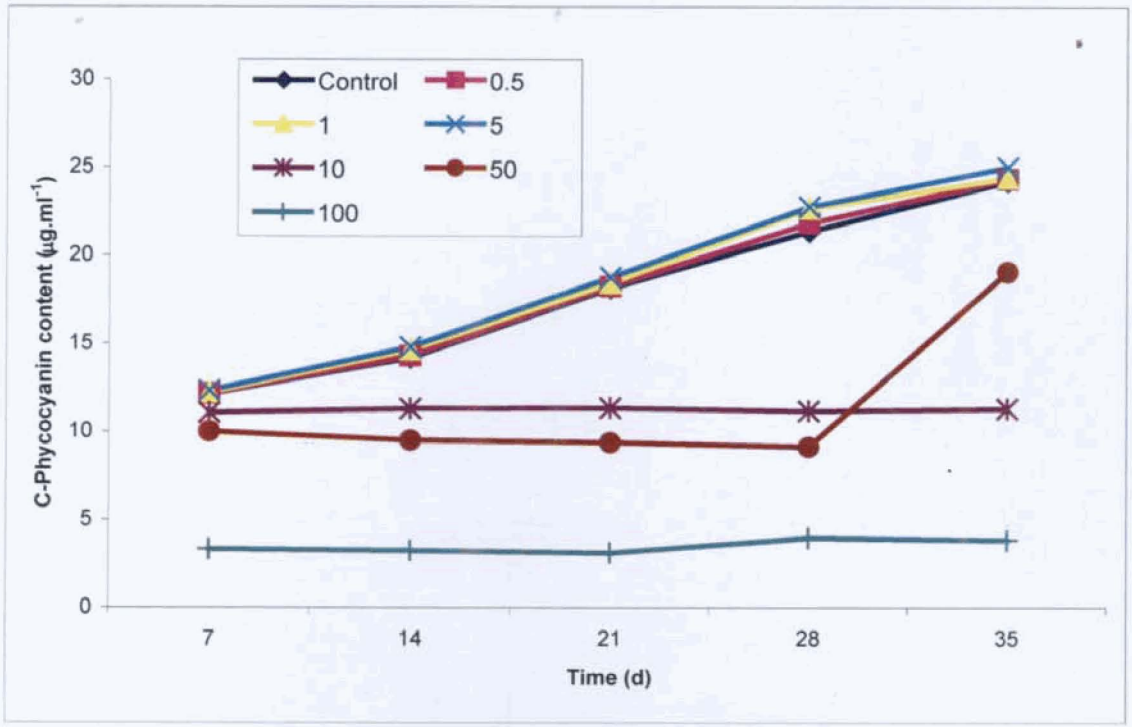


Fig.71 Effect of 2,4-D on c-phycocyanin content of *Westiellopsis prolifica* up to 35 days of incubation.

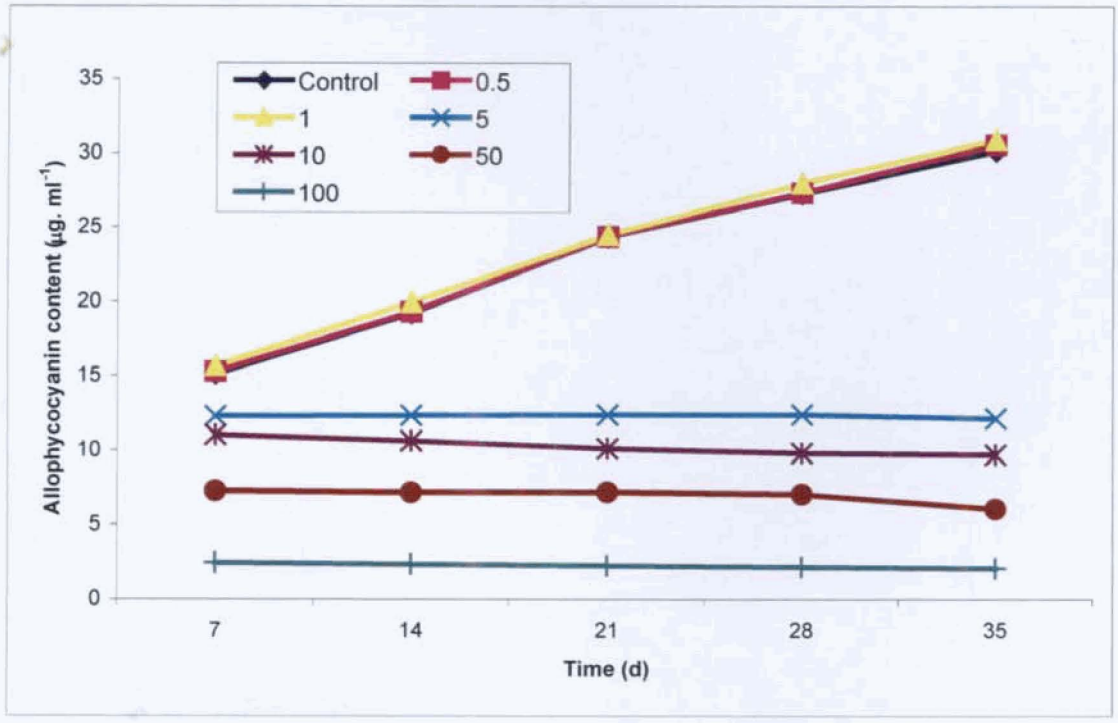


Fig.72 Effect of 2,4-D on allophycocyanin content of *Westiellopsis prolifica* up to 35 days of incubation.

Table 71. Effect of 2,4-D on phycobilin content c-phycoerythrin (μg.ml^{-1} culture) of *Westiellopsis prolifica* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	1.21 ± 0.09	1.54 ± 0.07	1.61 ± 0.09	1.72 ± 0.05	2.12 ± 0.06
0.5	1.22 ± 0.05	1.56 ± 0.09	1.62 ± 0.06	1.74 ± 0.04	2.13 ± 0.09
1	1.24 ± 0.08	1.57 ± 0.10	1.75 ± 0.07	1.92 ± 0.05	2.25 ± 0.05
5	1.12 ± 0.06	1.58 ± 0.09	1.81 ± 0.09	1.99 ± 0.09	2.29 ± 0.04
10	1.07 ± 0.09	0.84 ± 0.08	0.79 ± 0.06	0.77 ± 0.04	0.71 ± 0.05
50	0.82 ± 0.07	0.80 ± 0.09	0.75 ± 0.04	0.64 ± 0.05	0.54 ± 0.06
100	0.64 ± 0.06	0.51 ± 0.10	0.46 ± 0.03	0.41 ± 0.04	0.39 ± 0.09

Values represent mean of three replicates ± S.D.

Table 72. Effect of 2,4-D on phycobilin content c-phycocyanin (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	19.11 ± 0.05	20.27 ± 0.06	21.36 ± 0.05	22.41 ± 0.04	23.72 ± 0.07
0.5	19.14 ± 0.06	20.29 ± 0.08	21.38 ± 0.09	22.43 ± 0.05	23.81 ± 0.06
1	19.21 ± 0.04	20.34 ± 0.06	21.46 ± 0.08	22.54 ± 0.06	23.92 ± 0.05
5	18.34 ± 0.09	18.36 ± 0.07	18.41 ± 0.06	18.67 ± 0.05	18.52 ± 0.04
10	18.12 ± 0.09	18.14 ± 0.07	18.15 ± 0.09	17.24 ± 0.04	17.11 ± 0.06
50	17.26 ± 0.07	17.24 ± 0.06	17.21 ± 0.09	16.43 ± 0.09	16.27 ± 0.05
100	13.11 ± 0.09	13.12 ± 0.05	13.08 ± 0.06	12.94 ± 0.07	12.81 ± 0.04

Values represent mean of three replicates ± S.D.

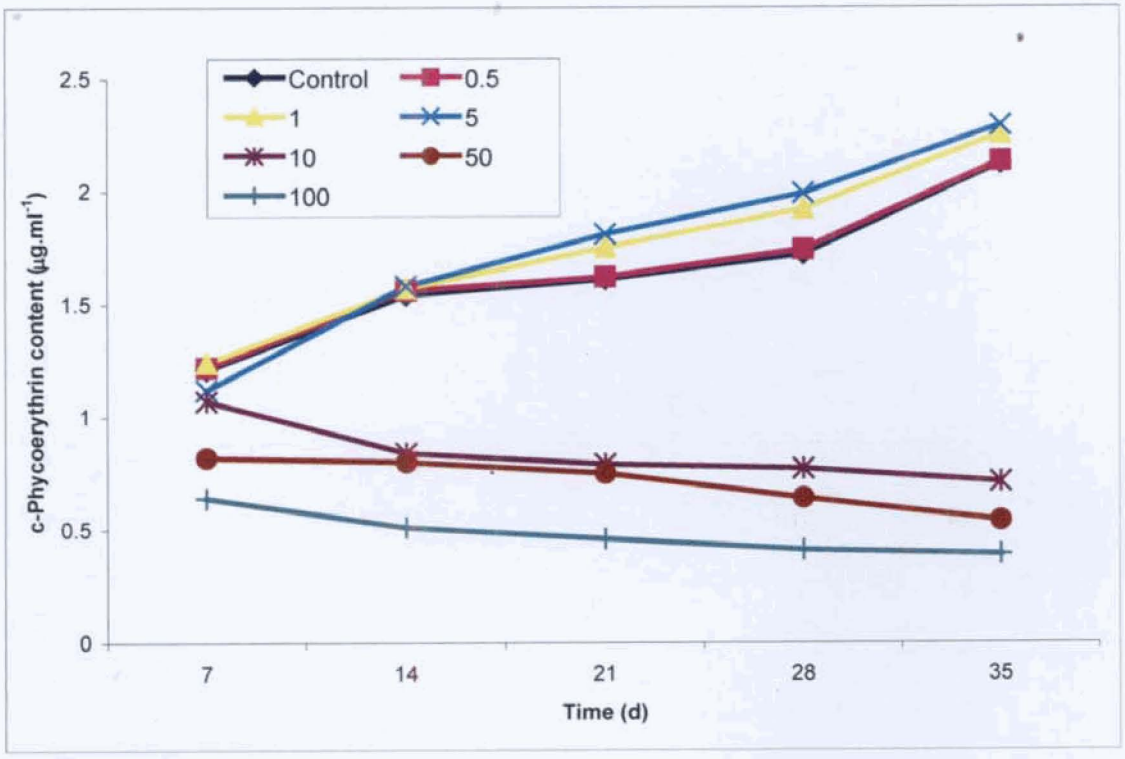


Fig.73 Effect of 2,4-D on c-phycoerythrin content of *Westiellopsis prolifica* up to 35 days of incubation.

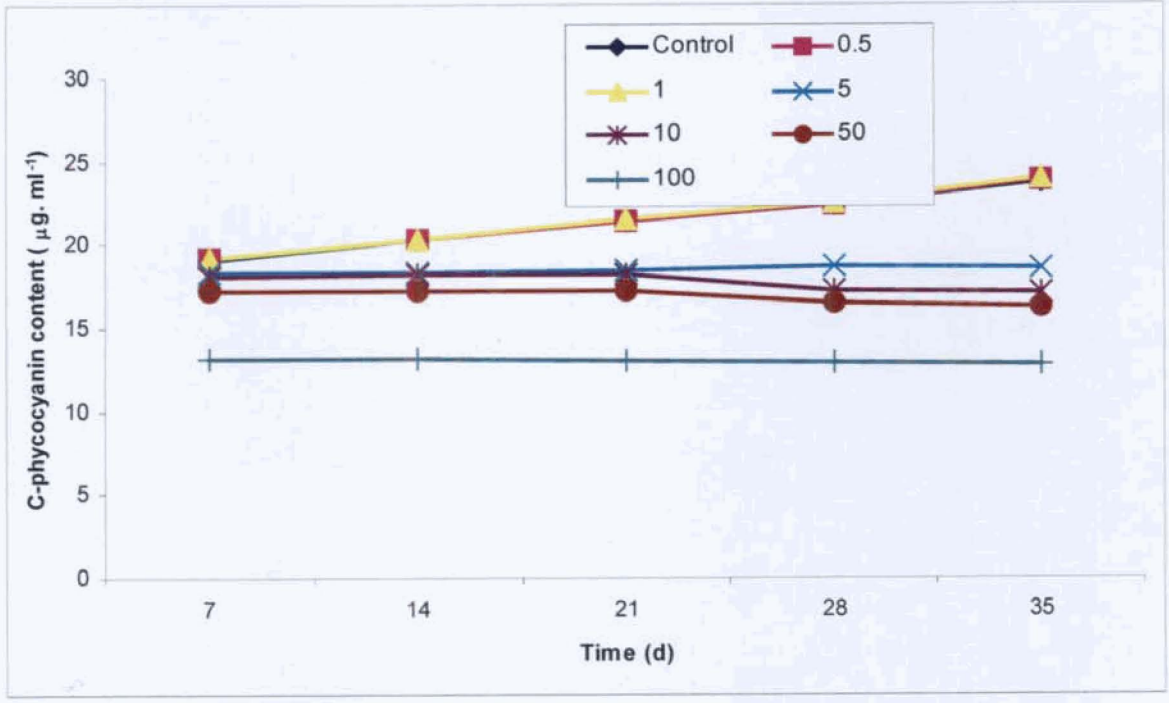


Fig.74 Effect of 2,4-D c-phycocyanin content of *Scytonema cincinnatum* up to 35 days of incubation.

Table 73. Effect of 2,4-D on phycobilin content allophycocyanin (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	17.17 ± 0.06	18.26 ± 0.05	18.95 ± 0.07	19.31 ± 0.05	20.42 ± 0.03
0.5	17.19 ± 0.04	18.29 ± 0.02	18.99 ± 0.05	19.52 ± 0.03	20.44 ± 0.07
1	17.21 ± 0.09	18.34 ± 0.06	19.34 ± 0.09	19.86 ± 0.11	20.47 ± 0.05
5	17.01 ± 0.06	17.15 ± 0.07	17.27 ± 0.10	17.14 ± 0.10	17.02 ± 0.04
10	16.73 ± 0.05	16.74 ± 0.08	16.24 ± 0.06	16.17 ± 0.09	16.09 ± 0.06
50	15.12 ± 0.04	15.13 ± 0.02	15.07 ± 0.11	14.84 ± 0.06	14.35 ± 0.05
100	12.37 ± 0.03	12.19 ± 0.03	12.01 ± 0.09	11.82 ± 0.05	11.51 ± 0.04

Values represent mean of three replicates ± S.D.

Table 74. Effect of 2,4-D on phycobilin content c-phycoerythrin (μg.ml^{-1} culture) of *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
Control	1.611 ± 0.05	1.624 ± 0.09	1.652 ± 0.04	1.661 ± 0.06	1.684 ± 0.09
0.5	1.614 ± 0.09	1.627 ± 0.06	1.659 ± 0.09	1.665 ± 0.07	1.689 ± 0.06
1	1.620 ± 0.11	1.632 ± 0.07	1.672 ± 0.10	1.676 ± 0.09	1.692 ± 0.05
5	1.602 ± 0.06	1.582 ± 0.05	1.583 ± 0.11	1.672 ± 0.07	1.562 ± 0.04
10	1.527 ± 0.06	1.529 ± 0.06	1.531 ± 0.08	1.521 ± 0.05	1.514 ± 0.05
50	1.511 ± 0.05	1.513 ± 0.09	1.510 ± 0.06	1.501 ± 0.04	1.424 ± 0.07
100	1.210 ± 0.08	1.207 ± 0.05	1.198 ± 0.05	1.172 ± 0.03	1.164 ± 0.06

Values represent mean of three replicates ± S.D.

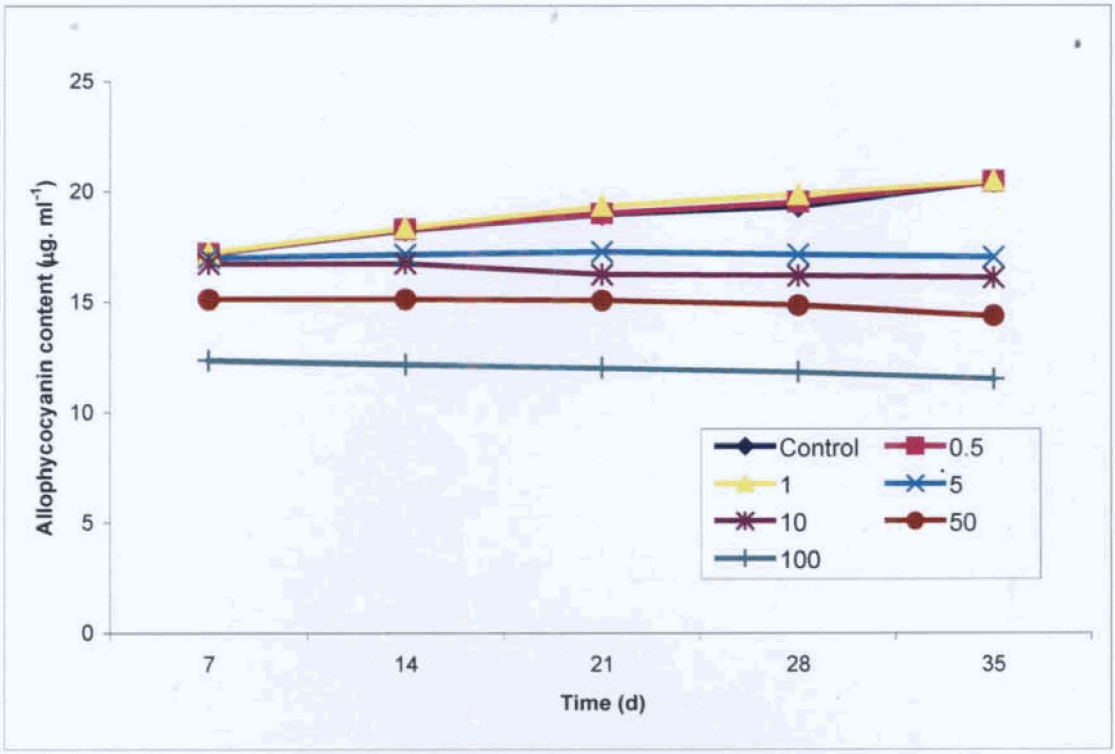


Fig.75 Effect of 2,4-D on allophycocyanin content of *Scytonema cincinnatum* up to 35 days of incubation.

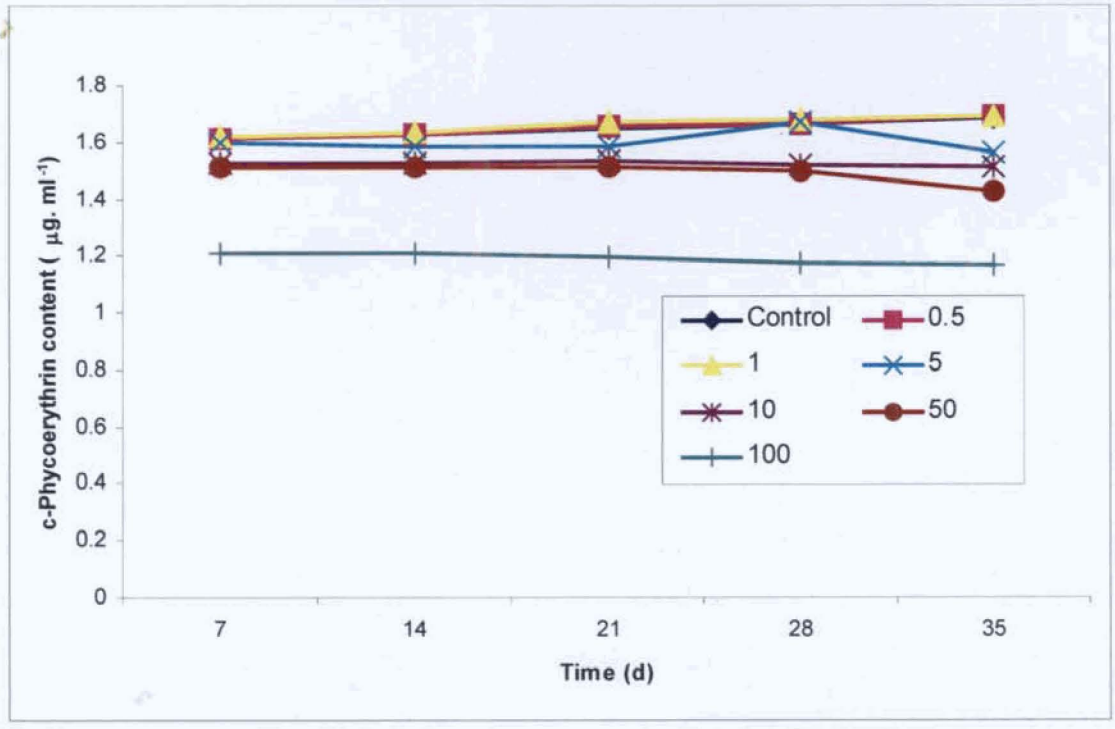


Fig.76 Effect of 2,4-D on c-phycoerythrin content of *Scytonema cincinnatum* up to 35 days of incubation.

Table 75. Effect of 2,4-D on ammonia excretion (n moles ml^{-1} culture) of *Westiellopsis prolifica* and *Scytonema cincinnatum* up to 35 days of incubation at 25 ± 1°C with 12 h light/dark cycle

Concentration (ppm)	Days after inoculation				
	7	14	21	28	35
W. prolifica					
Control	20.37 ± 0.15	93.42 ± 0.11	70.11 ± 0.11	48.26 ± 0.12	32.11 ± 0.13
0.5	21.34 ± 0.16	95.41 ± 0.19	72.14 ± 0.12	49.46 ± 0.13	34.01 ± 0.12
1	22.45 ± 0.19	99.27 ± 0.12	64.22 ± 0.15	40.27 ± 0.12	31.42 ± 0.11
5	24.72 ± 0.18	105.12 ± 0.13	65.72 ± 0.13	41.34 ± 0.11	35.71 ± 0.10
10	19.26 ± 0.14	52.12 ± 0.14	43.44 ± 0.12	38.26 ± 0.10	31.19 ± 0.12
50	18.41 ± 0.13	16.43 ± 0.11	12.71 ± 0.15	7.89 ± 0.19	6.94 ± 0.11
100	12.11 ± 0.12	11.42 ± 0.14	10.21 ± 0.14	7.67 ± 0.18	6.53 ± 0.10
S. cincinnatum					
Control	18.24 ± 0.15	83.41 ± 0.12	76.24 ± 0.10	35.21 ± 0.11	31.24 ± 0.11
0.5	19.26 ± 0.14	85.72 ± 0.11	63.11 ± 0.11	38.74 ± 0.16	25.91 ± 0.10
1	19.87 ± 0.10	89.26 ± 0.10	59.36 ± 0.12	44.62 ± 0.12	28.36 ± 0.12
5	16.24 ± 0.11	96.73 ± 0.12	55.27 ± 0.12	46.11 ± 0.11	35.43 ± 0.11
10	15.31 ± 0.14	15.45 ± 0.17	14.28 ± 0.13	12.17 ± 0.15	10.06 ± 0.13
50	13.46 ± 0.15	13.72 ± 0.12	14.14 ± 0.15	11.28 ± 0.12	5.64 ± 0.12
100	10.72 ± 0.10	10.96 ± 0.15	10.84 ± 0.13	5.74 ± 0.10	4.21 ± 0.13

Values represent mean of three replicates ± S.D.

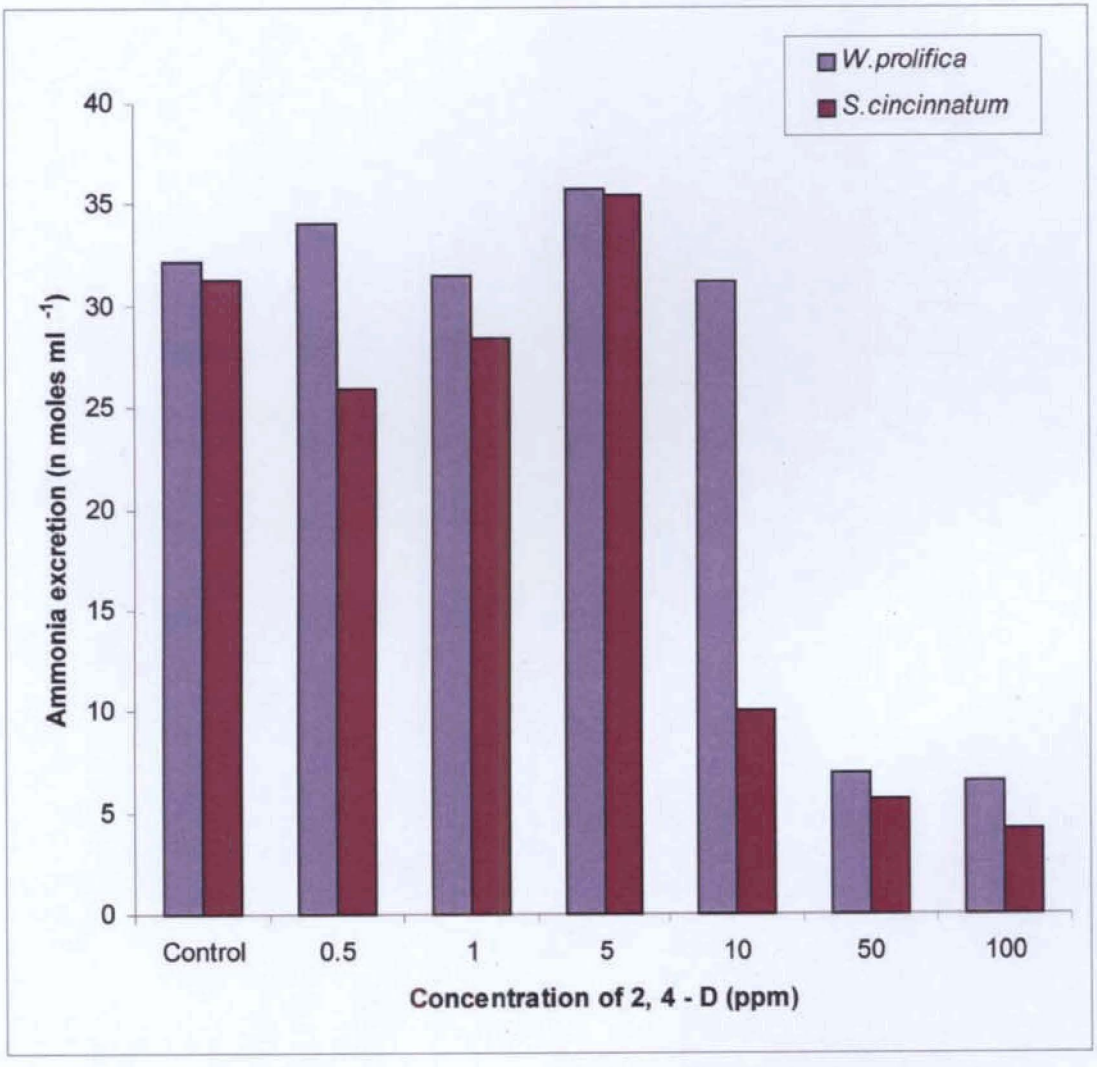


Fig.77 Effect of 2,4-D on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 35th day of incubation.

According to Khalil *et al.* (1980), *Chlamydomonas* and *Anabaena* can grow well in 25 ppm 2,4-D. A gradual reduction in total nitrogen fixed by cyanobacteria was observed with increase in the concentration of various herbicide. It agrees with the findings of Lundquist (1970), Da Silva *et al.* (1975), Das (1976), Roychoudhury and Kaushik (1986), Goyal *et al.* (1991) and Likhitkar and Tarar (1996). Tiwari *et al.* (1981) reported that 2,4-D induces growth and heterocyst formation in cyanobacteria but higher concentration affect the growth. Thus it was expected that 2,4-D at higher concentration may block carbondioxide fixation either by reducing photolysis of water or by interfering at the level of electron transport chain (Moreland, 1980).

The inhibitory effect on cyanobacterial growth has been reported for many herbicides (Padhy, 1985). This inhibition may be caused due to the primary effect of these herbicides at the photosynthesis level which then leads to several secondary effects. The site of action of herbicides inhibiting electron transport is closely associated with PSII. Therefore reactions coupled with PSII such as noncyclic electron transport with water as electron donor and various electron acceptors get inhibited. The severe depletion of phycobilins on addition of 2,4-D can also force the cyanobacterial cells to have only cyclic photophosphorylation resulting the cessation of carbondioxide fixation. This can limit the supply of carbon compounds which serve as the main source of energy and raw material for the synthesis of other cellular constituents.

There are reports that the physiological damage generated at higher doses of 2,4-D may be partially reduced by glucose or amino acids (Nanda and Padhi, 1992; Kaur *et al.*, 1997). Since the heterocyst development depends on the cyanobacterial growth, the herbicide concentration inhibiting growth also inhibits the heterocyst and akinete formation (Ahluwalia and Dahuja, 1997).

The depletion of cyanobacterial pigments seems to result from photooxidation induced by the inability of chlorophyll to dissipate its absorbed excitation energy due to inhibition of electron transport. The inhibition limits the availability of NADPH (Moreland, 1980). Since energy and reducing potential provided by NADPH play a vital role in many biosynthetic pathways, the changes in other parameters are also expected.

The possible utilization of phycobilin pigments by cyanobacterial cells under nitrogen stress caused by the 2,4-D results in reduction of protein level. These phytotoxic chemicals move into the cell affecting the electron transport and enzymatic activities resulting in the destruction of metabolic process (Lal and Saxena, 1980). According to Ahluwalia and Dahuja (1997), the release of ammonia at higher concentration of herbicide may be partly due to the death of cyanobacterial cells.

There are several findings on the toxic effect of herbicides to a large number of cyanobacteria, thus affecting the total productivity (Singh, 1973; Lal and Saxena, 1980; Padhy, 1985; Stratton, 1987; Kolte and Goyal, 1990; Venkataraman *et al.*, 1994). Herbicides reduce the growth, heterocyst

differentiation and nitrogen fixation of cyanobacteria (Kapoor and Sharma, 1980; Roychoudhury and Kaushik, 1986; Ahluwalia, 1988). Thus it is evident from the present study that *Westiellopsis prolifica* and *Scytonema cincinnatum* remain unaffected at low concentration of 2,4-D and thus this biological system can be effectively employed for nitrogen build up in paddy field soils.

4.8. EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM SOURCES ON THE GROWTH AND AMMONIA EXCRETION OF *WESTIELLOPSIS PROLIFICA* AND *SCYTONEMA CINCINNATUM*

4.8.1. Results

Cyanobacterial cultures grown in ammonium sulphate showed better growth rate than the cultures grown in urea (Table 76 & Fig. 78). Maximum growth produced by the cultures at 5 ppm of superphosphate suggest that it may be optimum level of phosphorus and even at low concentration the cyanobacterial cultures have the ability to utilize it completely and produce higher biomass (Table 77 & Fig. 79). In the case of potassium sources, the cyanobacterial cultures grown in potassium sulphate at 20 ppm showed maximum growth than the cultures grown in commercial potash (Table 78 & Fig. 80). The cyanobacterial cultures produced maximum protein at 5 ppm super phosphate than nitrogen and potassium sources (Table 79 & Fig. 81, 82). Among nitrogen and potassium sources, the production of protein was almost equal in both urea and ammonium sulphate and in potash and potassium sulphate respectively. Among nitrogen, phosphorus and

potassium sources, the production of chlorophyll-a was higher for ammonium sulphate, super phosphate and potassium sulphate respectively (Table 80 & Fig. 83, 84). Nitrogen and potassium sources showed maximum chlorophyll-a at 20 ppm while phosphorus sources showed maximum at 5 ppm superphosphate. Ammonia excretion was drastically suppressed in cultures grown in nitrogen sources and the excretion of ammonia was maximum by the cyanobacterial cultures grown in urea than ammonium sulphate (Table 81 & Fig. 85). The ammonia excretion was found to be maximum on 14^{th} day cultures in phosphate sources (Table 82 & Fig. 86). The ammonia excretion by potassium grown cultures was maximum at 21^{st} day of incubation and the excretion of ammonia was slightly higher in potassium sulphate than commercially available potash (Table 83 & Fig. 87).

4.8.2. Discussion

Cyanobacterial nitrogen fixation is one of the major components of the nitrogen cycle in the soil for sustenance of the nitrogen levels. The survival of the cyanobacteria along with rice crop in the rice fields demands a higher degree of adaptability to conditions like light availability, modification of nutrient levels and amendments of various agrochemicals.

The subterranean cyanobacterial flora is influenced by the type and mode of application of inorganic fertilizers such as nitrogen, phosphorus and potassium (Roger *et al.*, 1980). Among the nutrients disbursed through fertilizers in the fields, nitrogen plays an important role in sustaining crop

productivity because of high crop requirement and its universal deficiency in tropical soils.

According to the present investigation, the ammonia excretion was drastically suppressed in cultures grown in nitrogen sources and it agrees with the findings of Anand and Parameswaran (1992) and Amsaveni and Kannaiyan (1998). But enhancement of nitrogenase activity in the presence of exogenous ammonium source in *Anabaena* sp. CA was reported by Bottomley *et al.* (1979) and a strain *Cylindrospermum* showed nitrogenase activity in presence of nitrogenous fertilizers (Anand, 1990). Cyanobacteria can utilize all available sources of nitrogen in the form of organic and inorganic salts besides the elemental nitrogen. However, preference for nitrates (Myers and Kratz, 1955; Kaushik, 1986) or ammonium (Bottomley *et al.*, 1979; Ward and Wetzel, 1980) has been exhibited by several cyanobacteria. Anand (1992) reported that several cyanobacteria grow luxuriently in ammonia based commercial fertilizers. It has been reported that it is rarely possible to detect extracellular ammonia in nitrate or dinitrogen using cultures although the intracellular pool of ammonia is present even at the order of 1mM in cyanobacterial cultures (Rai *et al.*, 1984; Kerby *et al.*, 1986).

Ohmori *et al.* (1977) have reported that when supplied with both ammonia and nitrate as potential nitrogen source in growth medium, cyanobacteria first completely depleted ammonia in the medium and only then utilized nitrate. The cyanobacterial cultures grown in ammonium

sulphate showed better growth rate than the cultures grown in urea. It agrees with the findings of Kratz and Myers (1955) and Rawson (1985) that cyanobacteria mainly use inorganic nitrogen (ammonium or nitrate) to fulfil their nitrogen requirements but urea and other organic sources of nitrogen can also be assimilated. There are findings that nitrogen fertilizers showed a selective action on the different cyanobacterial strains (Subrahmanyan *et al.*, 1965c; Than Tun, 1969; Yoshida *et al.*, 1973).

In the present study, excretion of ammonia was greater in urea treated cyanobacterial cultures than ammonium sulphate treated cultures. There are reports that ammonium present in the growing medium did not allow the development of heterocyst (Anand and Karuppusamy, 1987) and it also inhibit the synthesis of nitrogenase in cultures that bear fully developed heterocyst. Thus ammonium seems to have a negative effect on nitrogenase synthesis besides the effect it has on heterocyst development. But a concentration dependent increase in growth and absorption of ammonium nitrogen by *Anabaena fertilissima* up to 50 ppm but at concentrations higher than 50 ppm, a reduction in nitrogen fixation was noticed (Stewart, 1964; Goyal and Marwaha, 1985). On the other hand, urea is a good nitrogen source for many cyanobacteria (Kratz and Myers, 1955). The urea application will prevent various losses like ammonia volatilization, denitrification, run off and leaching (Mikkelsen *et al.*, 1978; Savant *et al.*, 1983).

Table 76. Effect of nitrogen sources (urea and ammonium sulphate) on the growth (absorbance of the culture suspension at 760 nm) of cyanobacteria after 28 days of inoculation

Cyanobacteria	Urea treated cyanobacteria	% increase over control	$(NH_4)_2 SO_4$ treated cyanobacteria	% increase over control
Control				
S. cincinnatum	0.42 ± 0.09	--	0.42 ± 0.09	--
W. prolifica	0.51 ± 0.11	--	0.51 ± 0.11	--
1 ppm				
S. cincinnatum	0.62 ± 0.12	46.61	0.52 ± 0.11	23.80
W. prolifica	0.58 ± 0.11	13.72	0.64± 0.08	25.49
5 ppm				
S. cincinnatum	0.60 ± 0.13	42.85	0.58 ± 0.08	38.09
W. prolifica	0.64 ± 0.09	25.49	0.81 ± 0.11	58.82
10 ppm				
S. cincinnatum	0.60 ± 0.11	42.95	0.63± 0.09	50.00
W. prolifica	0.68 ± 0.12	33.33	0.86 ± 0.10	68.62
20 ppm				
S. cincinnatum	0.60 ± 0.09	42.85	0.71 ± 0.10	69.90
W. prolifica	0.81 ± 0.08	58.82	0.99 ± 0.09	94.11

Values represent mean of three replicates ± S.D.

Table 77. Effect of phosphorus source (commercial super phosphate) on the growth (absorbance of the culture suspension at 760 nm) of cyanobacteria after 28 days of inoculation

Cyanobacteria	Super phosphate treated cyanobacteria	% increase over control
Control		
S. cincinnatum	0.42 ± 0.09	--
W. prolifica	0.51 ± 0.11	--
1 ppm		
S. cincinnatum	0.76 ± 0.13	80.95
W. prolifica	0.08 ± 0.11	111.76
5 ppm		
S. cincinnatum	0.81 ± 0.12	92.85
W. prolifica	1.12 ± 0.10	119.60
10 ppm		
S. cincinnatum	0.74 ± 0.11	76.19
W. prolifica	1.06 ± 0.10	107.84
20 ppm		
S. cincinnatum	0.72 ± 0.09	71.42
W. prolifica	0.89 ± 0.10	74.50

Values represent mean of three replicates ± S.D.

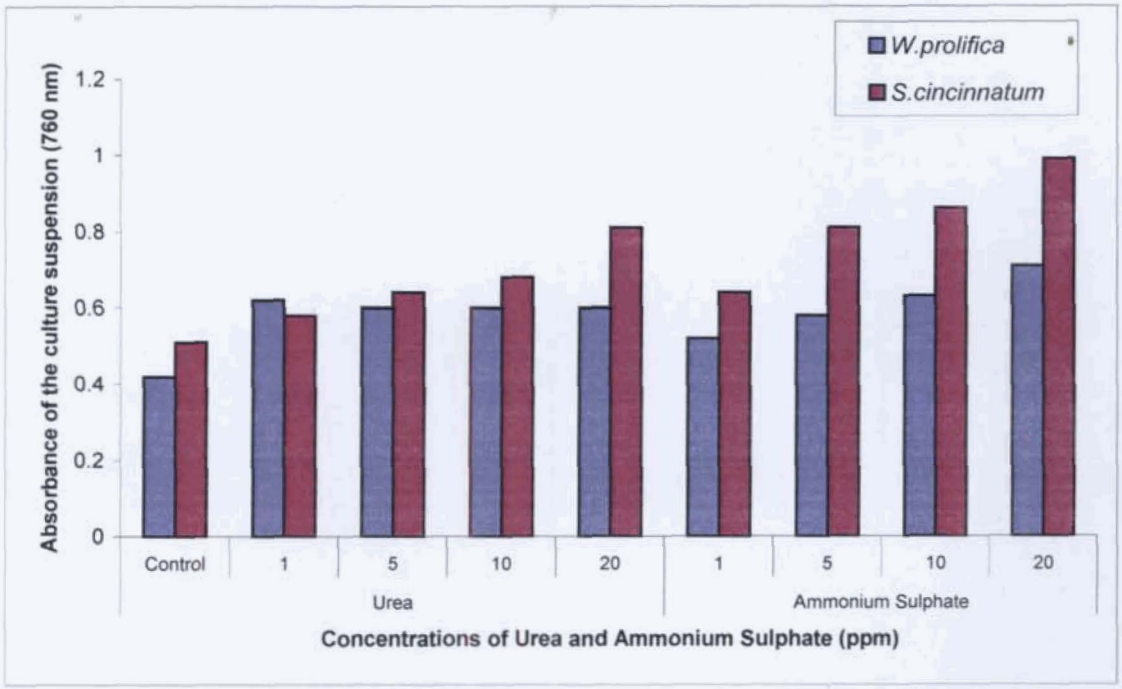


Fig.78 Effect of urea and ammonium sulphate on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 28th day of incubation.

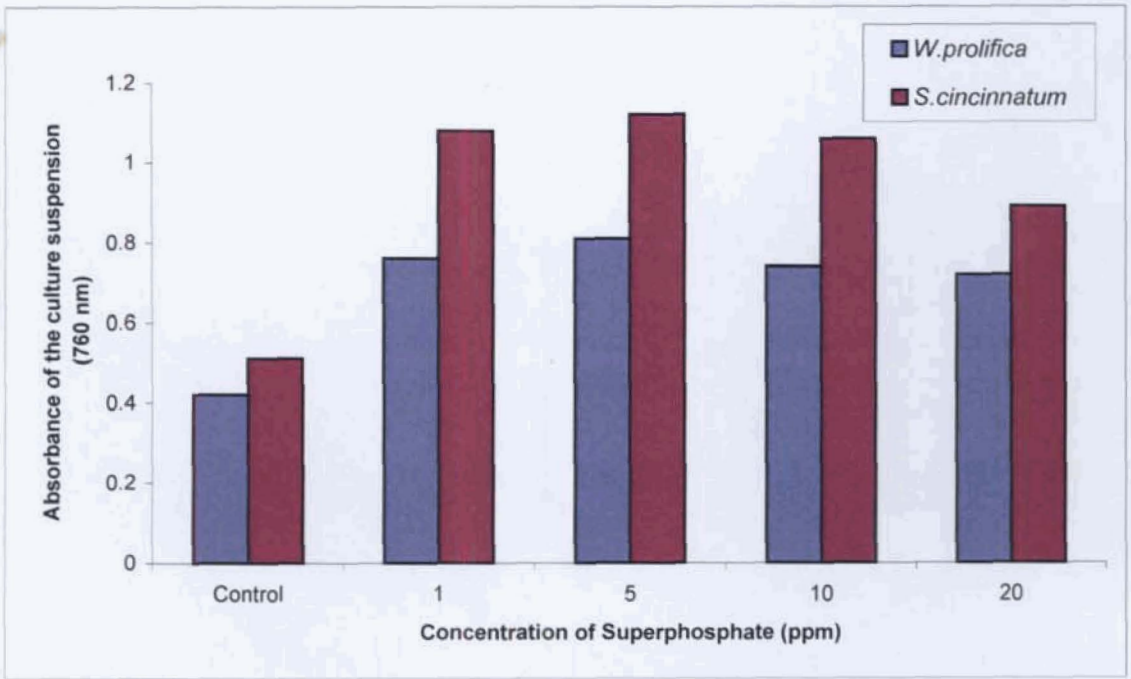


Fig.79 Effect of commercial superphosphate on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on 28th day of incubation.

Table 78. Effect of potassium sources (commercial potash and potassium sulphate on the growth (absorbance of the culture suspension at 760 nm) of cyanobacteria after 28 days of inoculation

	Potash treated cyanobacteria	% increase over control	K_2SO_4 treated cyanobacteria	% increase over control
Control				
S. cincinnatum	0.42 ± 0.09	--	0.42 ± 0.09	--
W. prolifica	0.51 ± 0.11	--	0.51 ± 0.11	--
1 ppm				
S. cincinnatum	0.45 ± 0.06	7.14	0.48 ± 0.05	14.28
W. prolifica	0.54 ± 0.08	5.88	0.57 ± 0.06	11.76
5 ppm				
S. cincinnatum	0.47 ± 0.07	11.90	0.52 ± 0.07	23.80
W. prolifica	0.58 ± 0.09	13.72	0.60 ± 0.06	17.64
10 ppm				
S. cincinnatum	0.50 ± 0.10	19.04	0.57 ± 0.10	35.71
W. prolifica	0.61 ± 0.07	19.60	0.65 ± 0.11	27.45
20 ppm				
S. cincinnatum	0.54 ± 0.05	28.57	0.62 ± 0.09	47.61
W. prolifica	0.69 ± 0.09	32.29	0.82 ± 0.07	68.67

Values represent mean of three replicates ± S.D.

Table 79. Effect of nitrogen, phosphorus and potassium sources on the protein content (μg.ml^{-1}) of *Scytonema cincinnatum* and *Westiellopsis prolifica* after 28 days of inoculation

	Urea	Ammonium sulphate	Super Phosphate	Potash	Potassium sulphate
Control					
Scytonema cincinnatum	73.46 ± 0.09	73.46 ± 0.09	73.46 ± 0.09	73.46 ± 0.09	73.46 ± 0.09
Westiellopsis prolifica	94.71 ± 0.11	94.71 ± 0.11	94.71 ± 0.11	94.71 ± 0.11	94.71 ± 0.11
1 ppm					
Scytonema cincinnatum	92.17 ± 0.07	95.79 ± 0.13	99.74 ± 0.11	75.32 ± 0.05	77.51 ± 0.11
Westiellopsis prolifica	99.37 ± 0.05	108.71 ± 0.11	141.39 ± 0.12	95.82 ± 0.03	99.85 ± 0.09
5 ppm					
Scytonema cincinnatum	93.08 ± 0.06	98.14 ± 0.12	105.12 ± 0.09	77.84 ± 0.06	79.88 ± 0.06
Westiellopsis prolifica	110.81 ± 0.11	133.46 ± 0.05	152.36 ± 0.07	98.31 ± 0.09	106.37 ± 0.09
10 ppm					
Scytonema cincinnatum	94.29 ± 0.12	98.45 ± 0.07	101.81 ± 0.05	78.22 ± 0.05	83.34 ± 0.08
Westiellopsis prolifica	115.43 ± 0.11	116.27 ± 0.09	150.34 ± 0.08	102.37 ± 0.08	108.49 ± 0.11
20 ppm					
Scytonema cincinnatum	94.86 ± 0.08	101.33 ± 0.06	97.14 ± 0.09	81.46 ± 0.09	89.53 ± 0.09
Westiellopsis prolifica	139.27 ± 0.09	141.11 ± 0.08	134.71± 0.08	109.27 ± 0.11	119.46 ± 0.12

Values represent mean of three replicates ± S.D.

Table 80. Effect of nitrogen, phosphorus and potassium sources on the chlorophyll-a content (μg. ml^{-1}) of *Scytonema cincinnatum* and *Westiellopsis prolifica* after 28 days of inoculation

	Urea	Ammonium sulphate	Super phosphate	Potash	Potassium sulphate
Control					
S. cincinnatum	7.82 ± 0.04	7.82 ± 0.04	7.82 ± 0.04	7.82 ± 0.04	7.82 ± 0.04
W. prolifica	8.94 ± 0.03	8.94 ± 0.03	8.94 ± 0.03	8.94 ± 0.03	8.94 ± 0.03
1 ppm					
S. cincinnatum	8.29 ± 0.02	8.12 ± 0.05	9.05 ± 0.06	7.84 ± 0.05	7.89 ± 0.07
W. prolifica	8.84 ± 0.05	9.21 ± 0.02	12.74 ± 0.05	9.45 ± 0.05	9.83 ± 0.06
5 ppm					
S. cincinnatum	8.21 ± 0.06	8.28 ± 0.03	9.31 ± 0.07	7.93 ± 0.04	7.96 ± 0.08
W. prolifica	9.35 ± 0.05	10.13 ± 0.03	12.98 ± 0.05	10.06 ± 0.03	10.01 ± 0.02
10 ppm					
S. cincinnatum	8.25 ± 0.07	8.56 ± 0.05	8.94 ± 0.04	8.18 ± 0.05	8.24 ± 0.05
W. prolifica	9.64 ± 0.08	10.39 ± 0.03	11.36 ± 0.08	10.13 ± 0.07	10.34 ± 0.03
20 ppm					
S. cincinnatum	8.19 ± 0.05	8.93 ± 0.04	7.84 ± 0.05	8.34 ± 0.08	8.36 ± 0.04
W. prolifica	11.14 ± 0.04	12.11 ± 0.03	10.91 ± 0.07	10.24 ± 0.06	11.37 ± 0.06

Values represent mean of three replicates ± S.D.

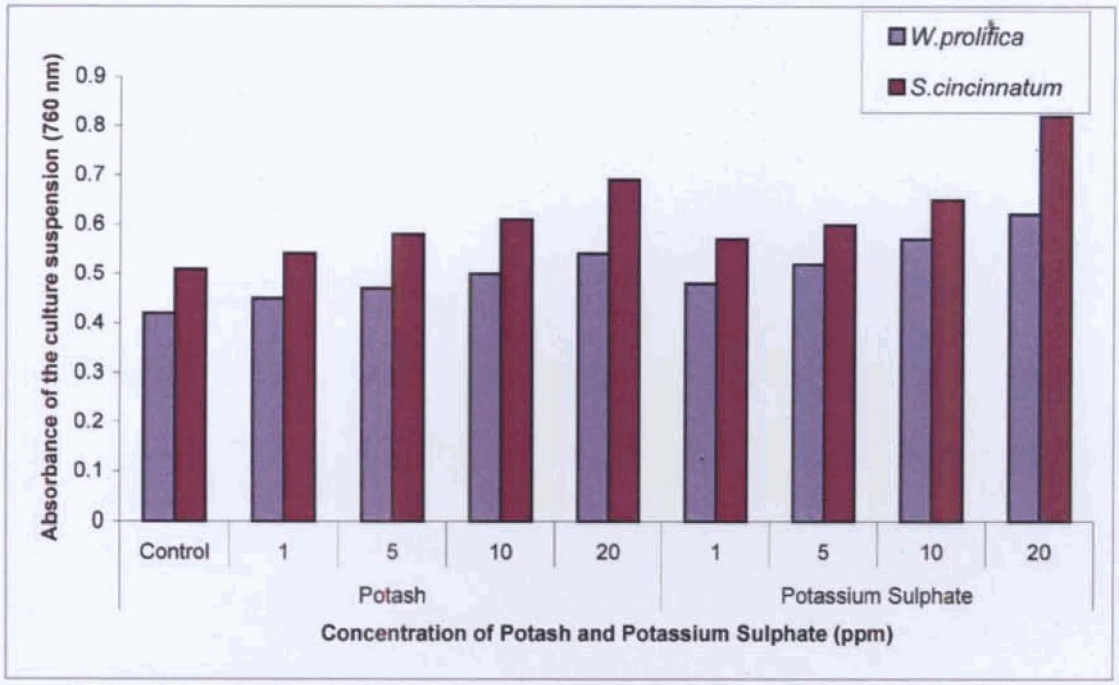


Fig.80 Effect of potash and potassium sulphate on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* and *Scytonema cincinnatum* on the 28th day of incubation.

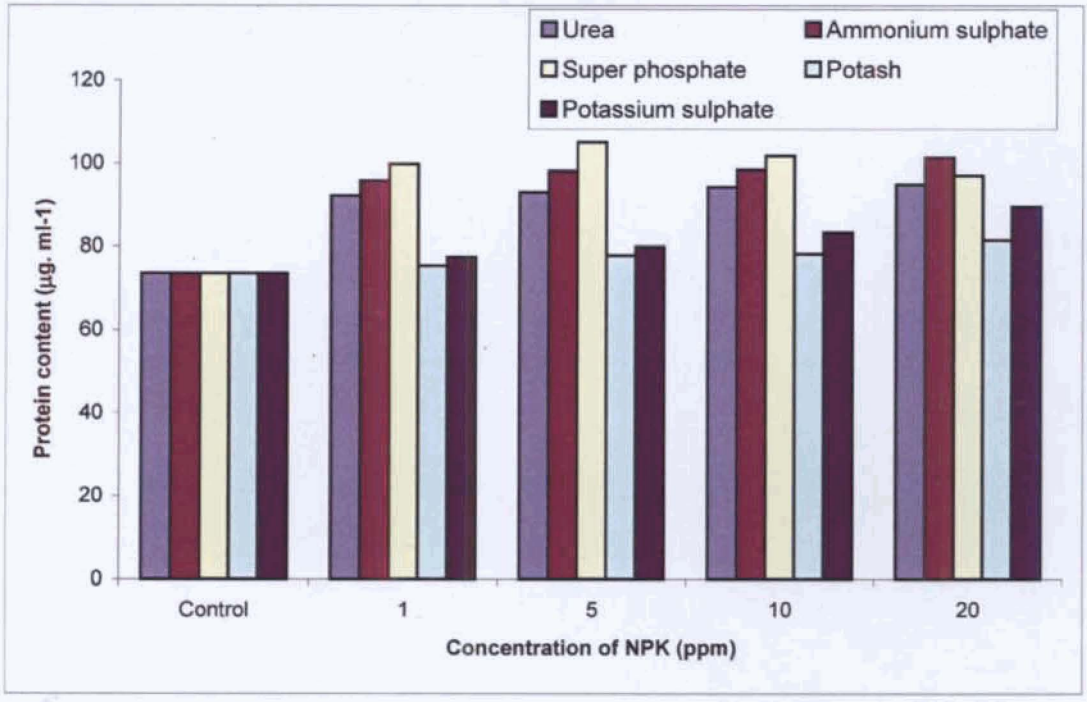


Fig.81 Effect of NPK sources on the protein content of *Scytonema cincinnatum* on 28th day of incubation.

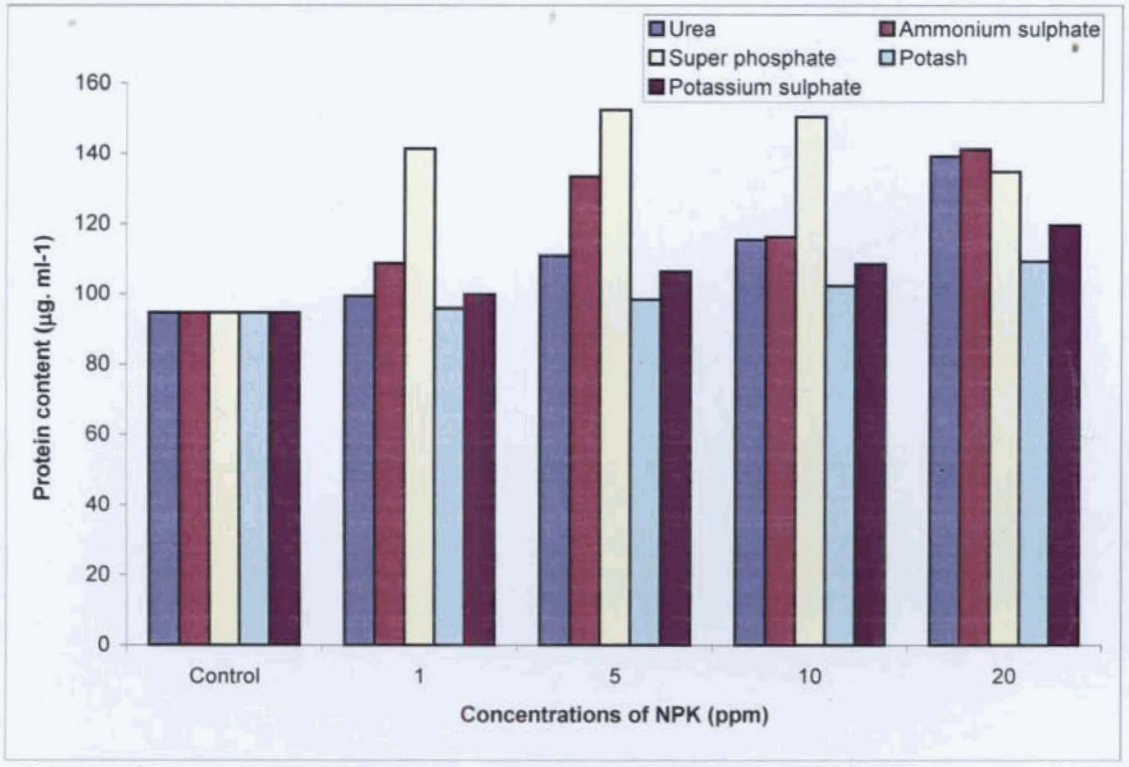


Fig.82 Effect of NPK sources on protein content of *Westiellopsis prolifica* on the 28th day of incubation.

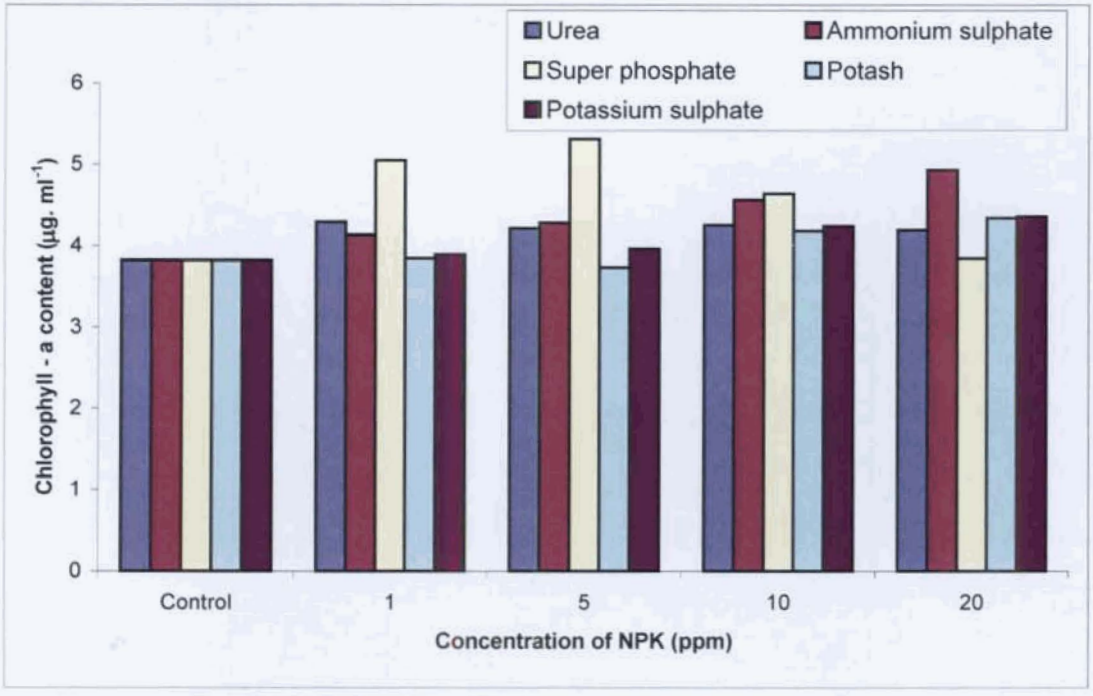


Fig.83 Effect of NPK sources on chlorophyll-a content of *Scytonema cincinnatum* on 28th day of incubation.

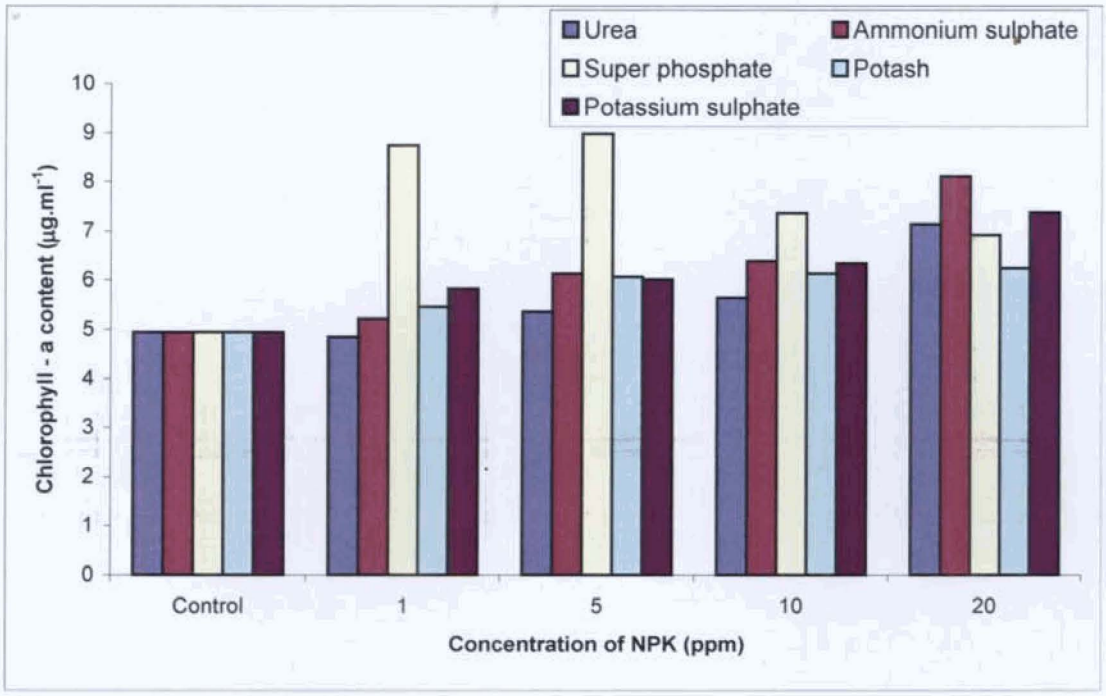


Fig.84 Effect of NPK sources on the chlorophyll-a content of *Westiellopsis prolifica* on 28th day of incubation.

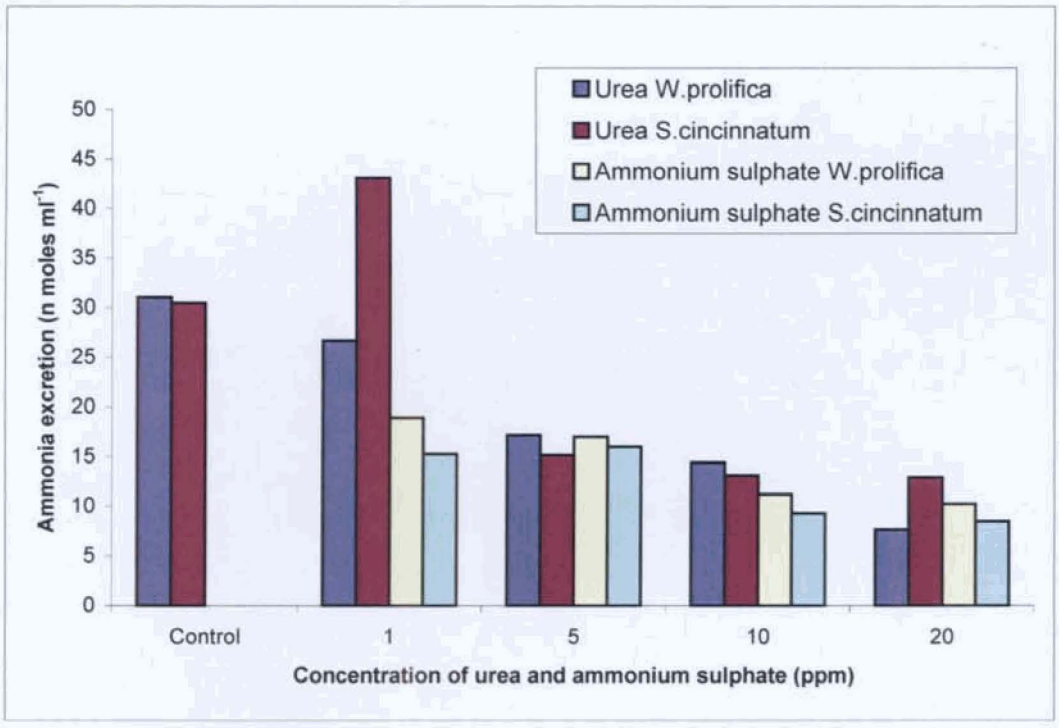


Fig.85 Effect of urea and ammonium sulphate on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 28th day of incubation.

Table 81. Effect of nitrogen sources (urea and ammonium sulphate) on ammonia excretion (n moles ml^{-1}) by *Scytonema cincinnatum* and *Westiellopsis prolifica* up to 28 days of inoculation

Concentration	Days after inoculation			
	7	14	21	28
Control				
S. cincinnatum	26.33 ± 0.09	87.14 ± 0.11	71.82 ± 0.09	30.46 ± 0.08
W. prolifica	30.12 ± 0.15	96.45 ± 0.10	78.37 ± 0.08	31.06 ± 0.09
1 ppm urea				
S. cincinnatum	19.43 ± 0.11	66.13 ± 0.12	54.25 ± 0.14	43.08 ± 0.09
W. prolifica	22.38 ± 0.08	72.11 ± 0.011	49.24 ± 0.05	26.72 ± 0.06
5 ppm urea				
S. cincinnatum	15.26 ± 0.14	43.41 ± 0.10	28.63 ± 0.06	15.17 ± 0.05
W. prolifica	18.78 ± 0.06	54.67 ± 0.08	36.44 ± 0.08	17.19 ± 0.11
10 ppm urea				
S. cincinnatum	12.46 ± 0.11	37.31 ± 0.07	16.81 ± 0.11	13.12 ± 0.12
W. prolifica	15.34 ± 0.10	45.47 ± 0.05	21.33 ± 0.07	14.41 ± 0.10
20 ppm urea				
S. cincinnatum	11.91 ± 0.07	18.31 ± 0.11	10.54 ± 0.10	12.93 ± 0.11
W. prolifica	13.22 ± 0.09	19.83 ± 0.06	15.72 ± 0.04	7.65 ± 0.07
1 ppm $(NH_4)_2 SO_4$				
S. cincinnatum	17.45 ± 0.09	43.27 ± 0.10	34.20 ± 0.09	15.25 ± 0.10
W. prolifica	19.37 ± 0.08	54.32 ± 0.09	36.78 ± 0.07	18.91 ± 0.06
5 ppm $(NH_4)_2 SO_4$				
S. cincinnatum	15.22 ± 0.06	23.31 ± 0.10	14.12 ± 0.08	16.02 ± 0.09
W. prolifica	16.71 ± 0.10	25.36 ± 0.11	23.11 ± 0.06	17.03 ± 0.10
10 ppm $(NH_4)_2 SO_4$				
S. cincinnatum	11.32 ± 0.08	18.45 ± 0.09	15.23 ± 0.07	9.29 ± 0.11
W. prolifica	14.09 ± 0.09	20.31 ± 0.07	16.12 ± 0.05	11.23 ± 0.13
20 ppm $(NH_4)_2 SO_4$				
S. cincinnatum	10.14 ± 0.10	17.26 ± 0.11	13.02 ± 0.11	8.46 ± 0.12
W. prolifica	12.93 ± 0.12	19.18 ± 0.05	14.36 ± 0.09	10.24 ± 0.10

Values represent mean of three replicates ± S.D.

Table 82. Effect of phosphorus sources (commercial super phosphate on ammonia excretion (n moles ml^{-1}) of *Scytonema cincinnatum* and *Westiellopsis prolifica* up to 28 days of inoculation

Concentration	Days after inoculation			
	7	14	21	28
Control				
S. cincinnatum	26.33 ± 0.09	87.14 ± 0.11	71.82 ± 0.09	30.46 ± 0.08
W. prolifica	30.12 ± 0.15	96.45 ± 0.10	78.37 ± 0.08	31.06 ± 0.09
1 ppm super phosphate				
S. cincinnatum	38.27 ± 0.11	71.49 ± 0.09	68.31 ± 0.10	40.26± 0.09
W. prolifica	40.01 ± 0.09	90.24 ± 0.09	81.36 ± 0.11	53.71 ± 0.09
5 ppm super phosphate				
S. cincinnatum	44.21 ± 0.12	94.32 ± 0.41	90.24 ± 0.12	49.14 ± 0.06
W. prolifica	48.46 ± 0.14	131.09 ± 0.13	99.36 ± 0.13	82.91 ± 0.11
10 ppm super phosphate				
S. cincinnatum	37.24 ± 0.13	78.37 ± 0.12	74.45 ± 0.14	42.41 ± 0.13
W. prolifica	42.83 ± 0.15	109.09 ± 0.10	91.36 ± 0.10	73.26 ± 0.12
20 ppm super phosphate				
S. cincinnatum	33.41 ± 0.16	81.07 ± 0.10	63.22 ± 0.09	30.02 ± 0.11
W. prolifica	39.74 ± 0.09	93.78 ± 0.11	86.47 ± 0.08	64.83 ± 0.10

Values represent mean of three replicates ± S.D.

Table 83. Effect of potassium sources (commercial potash and potassium sulphate) on ammonia excretion (n moles ml^{-1}) by *Scytonema cincinnatum* and *Westiellopsis prolifica* up to 28 days of inoculation

Concentration	Days after inoculation			
	7	14	21	28
Control				
S. cincinnatum	26.33 ± 0.09	87.14 ± 0.11	71.82 ± 0.09	30.46 ± 0.08
W. prolifica	30.12 ± 0.15	96.45 ± 0.10	78.37 ± 0.08	31.06 ± 0.09
1 ppm potash				
S. cincinnatum	31.92 ± 0.10	42.81 ± 0.07	88.44 ± 0.08	39.42 ± 0.05
W. prolifica	32.16 ± 0.07	46.34 ± 0.10	96.89 ± 0.08	45.14 ± 0.04
5 ppm potash				
S. cincinnatum	28.14 ± 0.09	48.27 ± 0.11	90.63 ± 0.09	41.75 ± 0.07
W. prolifica	33.71 ± 0.06	51.64 ± 0.10	98.37 ± 0.06	46.17 ± 0.09
10 ppm potash				
S. cincinnatum	29.42 ± 0.10	50.31 ± 0.06	92.18 ± 0.10	44.68 ± 0.06
W. prolifica	31.63 ± 0.11	54.11 ± 0.05	109.16 ± 0.09	51.34 ± 0.08
20 ppm potash				
S. cincinnatum	32.33 ± 0.09	45.39 ± 0.10	95.73 ± 0.06	50.07 ± 0.04
W. prolifica	35.45 ± 0.08	51.26 ± 0.05	102.46 ± 0.08	53.17 ± 0.05
1 ppm K_2SO_4				
S. cincinnatum	31.11 ± 0.12	45.71 ± 0.05	90.66 ± 0.07	41.06 ± 0.05
W. prolifica	32.85 ± 0.06	49.46 ± 0.04	93.41 ± 0.09	43.95 ± 0.05
5 ppm K_2SO_4				
S. cincinnatum	32.78 ± 0.06	45.62 ± 0.09	92.77 ± 0.08	52.67 ± 0.07
W. prolifica	33.91 ± 0.05	48.54 ± 0.07	99.73 ± 0.10	58.37 ± 0.09
10 ppm K_2SO_4				
S. cincinnatum	31.56± 0.05	49.02 ± 0.06	95.83 ± 0.10	66.72 ± 0.07
W. prolifica	35.19 ± 0.09	53.41 ± 0.11	109.94 ± 0.11	76.43 ± 0.06
20 ppm K_2SO_4				
S. cincinnatum	35.78 ± 0.12	50.58 ± 0.06	99.63 ± 0.10	70.16 ± 0.05
W. prolifica	38.42 ± 0.06	58.73 ± 0.04	112.45 ± 0.06	78.74 ± 0.09

Values represent mean of three replicates ± S.D.

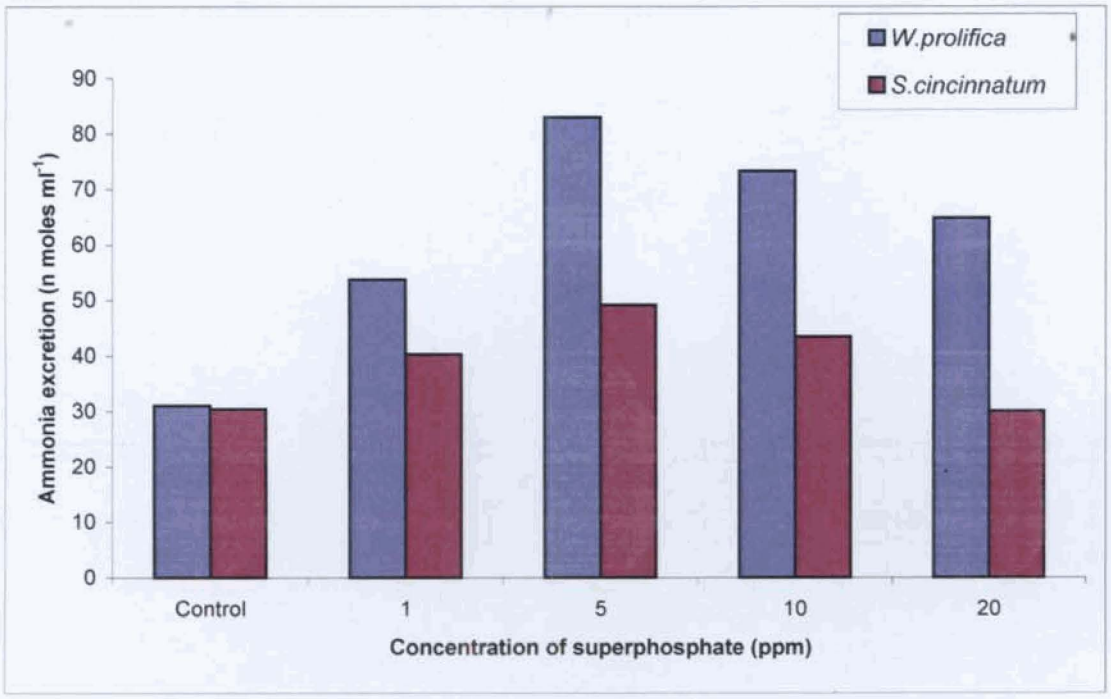


Fig.86 Effect of commercial superphosphate on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 28th day of incubation.

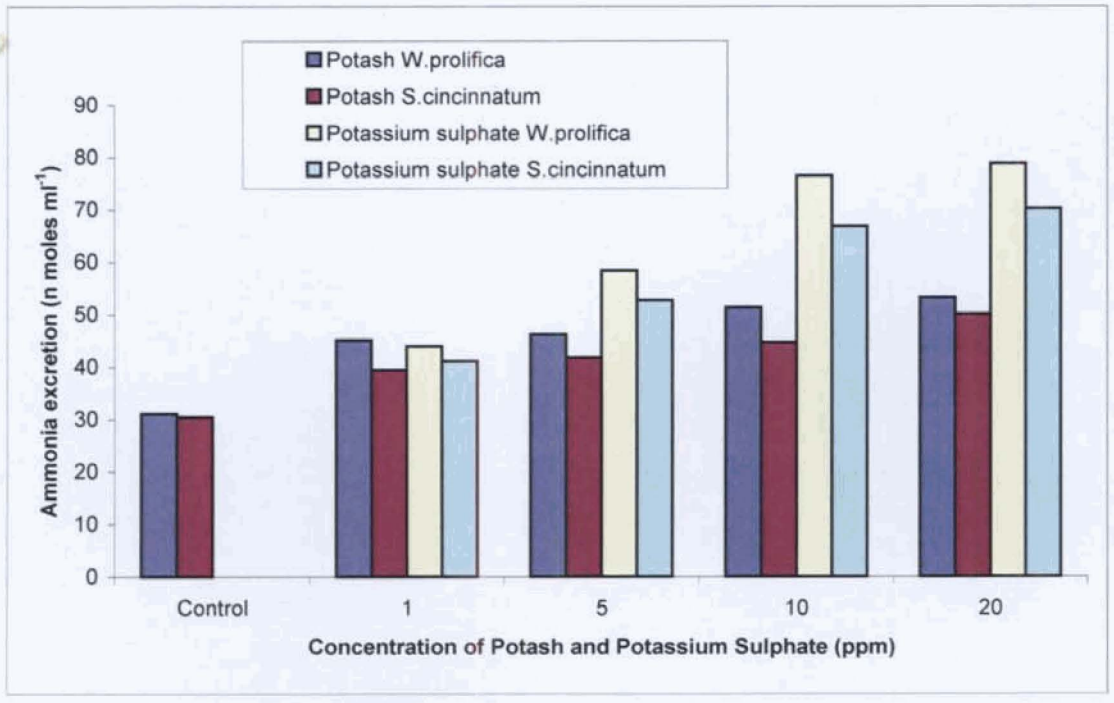


Fig.87 Effect of potash and potassium sulphate on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 28th day of incubation.

Urea appears to be taken up intact and actively by the cells and then metabolized intracellularly (Healy, 1977). It has been showed that for diazotrophic cyanobacteria, the tolerance limit for combined nitrogen with respect to growth was much higher than for nitrogen fixation (Goyal, 1989).

The present study reveals that maximum biomass production was showed by the cyanobacterial cultures grown in super phosphate. It agrees with the findings of Kannaiyan *et al.* (1992c) that a better biomass production of salt tolerant cyanobacterial cultures grown in presence of single super phosphate than in rock phosphate. In the present investigation the production of protein was higher in super phosphate treated cultures than that of potassium and nitrogen treated cultures. According to Prasad and Kashyap (1991), *Nostoc calcicola* grown under phosphate deficient condition showed a decrease in specific growth rate, phycocyanin content (50%), oxygen evolution and reduced ATP pool. Singh *et al.* (1994) investigated that protein content of *Nostoc calcicola* cells were decreased by 7% in phosphate starved cells.

Results of several studies showed that the phosphate application stimulates cyanobacterial growth and photo dependent biological nitrogen fixation (De and Biswas, 1952; De and Mandal, 1956; Wilson and Alexander, 1979; Roger and Kulasooriya, 1980). According to Stewart *et al.* (1971) there are blooms of nitrogen fixing cyanobacteria develop in aquatic system when phosphorous is available and combined nitrogen level is low. The phosphate levels in the aquatic systems have been related to cyanobacterial

growth and nitrogen fixation (Vanderhoef *et al.*, 1974; Baral and Kumar, 1994). The phosphate requirement of cyanobacteria was about 40 to 80 kg P_2O_5 per hectre in Indian Paddy fields. Application of 40 kg P_2O_5 per hectre increased the yield of *Aulosira, Aphanotheca* and *Gleotrichia* by 2.5, 3.5 and 5 times respectively. The availability of phosphate in the soil was greatly limited by soil pH and split application of phosphate was better than one basal application (Bisoyi and Singh, 1998). According to Healy (1973), phosphate uptake in *Anabaena variabilis* increased with temperature up to 35°C. There are reports that the rate of phosphate uptake was optimal between pH 7.5 – 9 and declined sharply in acidic range (Ullrich – Eberius and Yingchol, 1974; Lawry and Jensen, 1979) and also fell at alkaline conditions (Ritchie *et al.*, 1997).

According to the present study, in the case of potassium sources, the cyanobacterial cultures grown in potassium sulphate (20 ppm) showed maximum growth than the cultures grown in potash. The sulphate present in potassium sulphate might be the reason for increasing the growth of cyanobacterial cultures and it agrees with the findings of Amsaveni and Kannaiyan (1998). The potassium sources excreted maximum ammonia on 21st day in cultures and this lag in producing ammonia may be the adaptation time for cyanobacteria to K^+ ions which might have delayed this process of maximum ammonia excretion.

According to Venkataraman (1981) and Kannaiyan (1985) a combination of cyanobacteria and different commercial fertilizers have

shown that, up to 30% of the input of chemical fertilizers could be conserved in rice fields.

4.9. EFFECT OF IMMOBILIZATION IN DIFFERENT MEDIA ON PIGMENT, PROTEIN AND AMMONIA EXCRETION OF CYANOBACTERIA

4.9.1. Results

The media composition significantly influence the growth, pigment, protein content and ammonia excretion by the two cyanobacterial cultures. Among the different media, the nitrogen free BG-11 favoured maximum chlorophyll-a, protein and ammonia excretion (Table 84-86 & Fig. 88-93). The present findings showed that the growth was more in immobilized cultures than in control cultures.

The growth and ammonia excretion was significantly lower in the cyanobacteria immobilized in Allen and Arnon's nitrogen free medium and in Fogg's medium. However cyanobacteria cannot be immobilized in Allen and Arnon's N^+ medium because the alginate beads gets disintegrated after 2 or 3 days of inoculation. The BG-11 N^+ medium also showed signs of bead disintegration after one or two months of inoculation. In Fogg's medium, after two months of growth cyanobacteria get separated from the beads. So the best medium for immobilization of cyanobacteria was nitrogen free BG-11 medium. The cyanobacteria grows very fast in nitrogen free BG-11 medium and could be preserved for more than a year in intact beads without loosing the viability of cells (Plate 4, 5).

Table 84. Effect of immobilization in different media on chlorophyll-a content (μg. ml^{-1} culture) of *Westiellopsis prolifica* and *Scytonema cincinnatum* after 2 months of incubation at 25 ± 1°C with 12 h light/dark cycle

Culture Media	*Westiellopsis prolifica*		*Scytonema cincinnatum*	
	Control	Immobilized	Control	Immobilized
BG-11 N^+	4.72 ± 0.06	4.92 ± 0.08	4.01 ± 0.07	4.57 ± 0.09
BG-11 N^-	5.84 ± 0.07	7.06 ± 0.06	4.98 ± 0.07	5.23 ± 0.08
Allen & Arnon's N^-	3.50 ± 0.05	3.81 ± 0.07	2.96 ± 0.09	3.46 ± 0.08
Fogg's	3.71 ± 0.06	4.21 ± 0.06	3.11 ± 0.08	3.92 ± 0.06

Values represent mean of three replicates ± S.D.

Table 85. Effect of immobilization in different media on protein content (μg. ml^{-1} culture) of *Westiellopsis prolifica* and *Scytonema cincinnatum* after 2 months of incubation at 25 ± 1°C with 12 h light/dark cycle

Culture Media	*Westiellopsis prolifica*		*Scytonema cincinnatum*	
	Control	Immobilized	Control	Immobilized
BG-11 N^+	61.66 ± 0.19	85.83 ± 0.23	56.01 ± 0.18	75.36 ± 0.23
BG-11 N^-	81.01 ± 0.23	91.66 ± 0.24	73.26 ± 0.19	84.16 ± 0.25
Allen & Arnon's N^-	32.53 ± 0.21	58.33 ± 0.20	35.31 ± 0.23	41.31 ± 0.23
Fogg's	36.41 ± 0.23	65.83 ± 0.19	34.16 ± 0.21	53.67 ± 0.21

Values represent mean of three replicates ± S.D.

Table 86. Effect of immobilization in different media on ammonia excretion (n. moles ml^{-1}) of *Westiellopsis prolifica* and *Scytonema cincinnatum* after 2 months of incubation at 25 ± 1°C with 12 h light/dark cycle

Culture Media	*Westiellopsis prolifica*		*Scytonema cincinnatum*	
	Control	Immobilized	Control	Immobilized
BG-11 N^+	23.05 ± 0.09	31.36 ± 0.11	20.16 ± 0.08	27.98 ± 0.13
BG-11 N^-	36.31 ± 0.11	42.46 ± 0.12	31.42 ± 0.09	38.16 ± 0.11
Allen & Arnon N^-	14.26 ± 0.10	20.91 ± 0.13	15.17 ± 0.09	20.09 ± 0.12
Fogg's	18.21 ± 0.08	28.19 ± 0.10	23.29 ± 0.11	26.19 ± 0.10

Values represent mean of three replicates ± S.D.

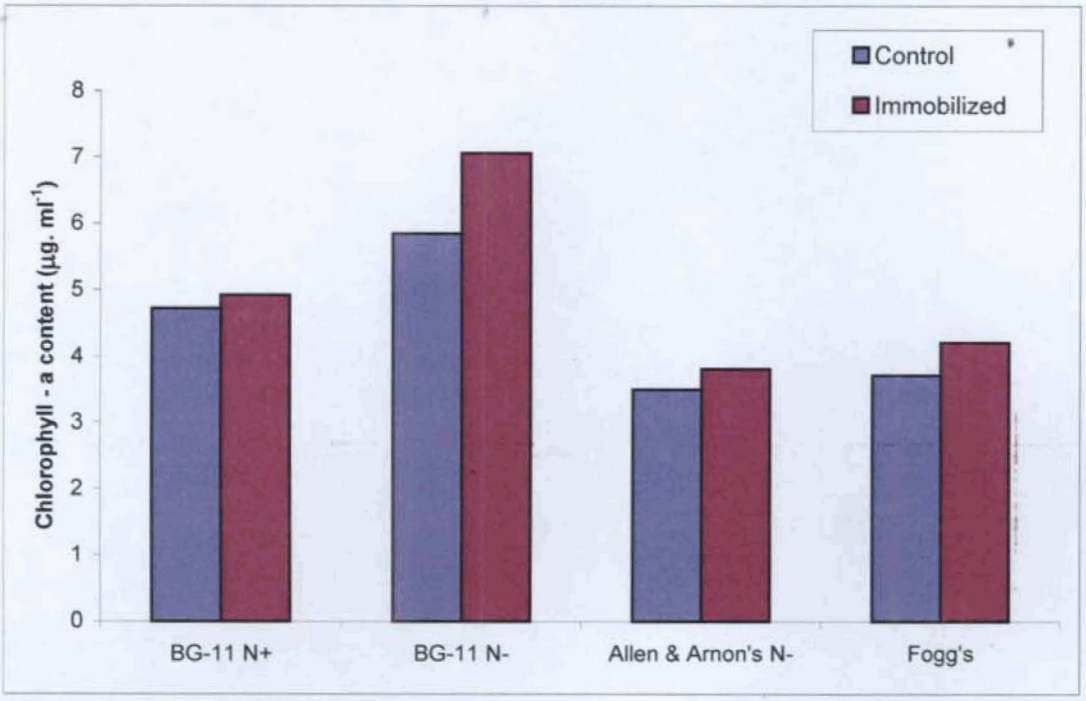


Fig.88 Effect of immobilization on chlorophyll-a content of *Westiellopsis prolifica* after two months of incubation.

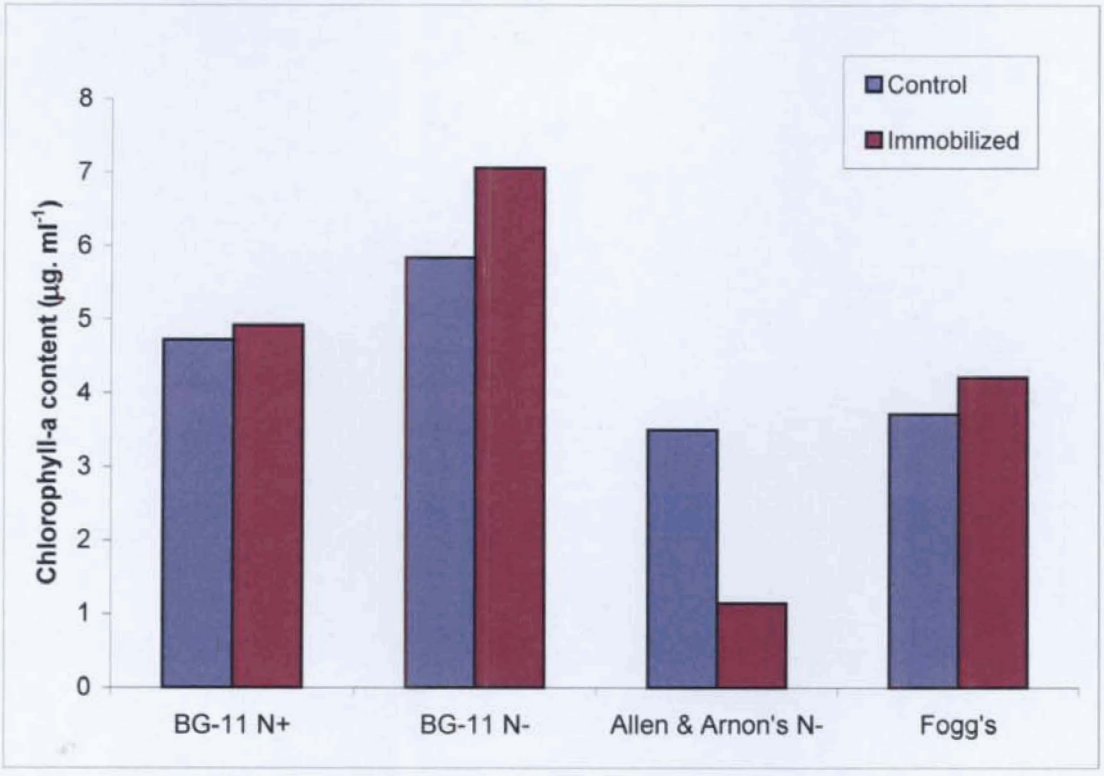


Fig.89 Effect of immobilization on chlorophyll-a content of *Scytonema cincinnatum* after two months of incubation.

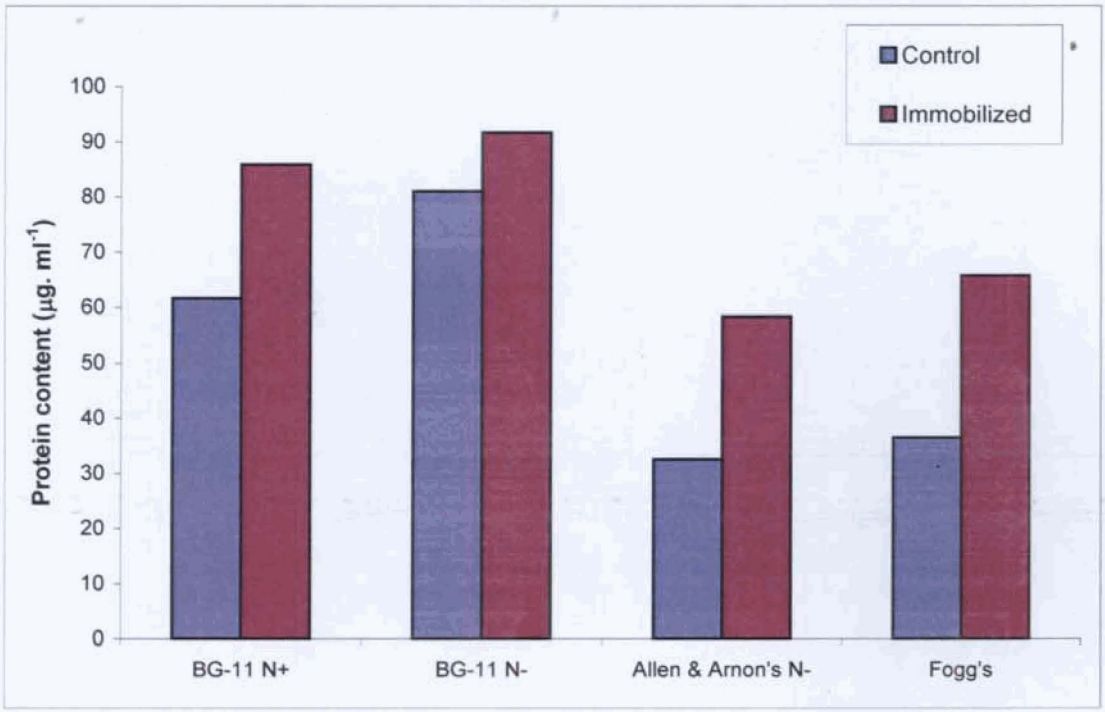


Fig.90 Effect of immobilization on the protein content of *Westiellpsis prolifica* after two months of incubation.

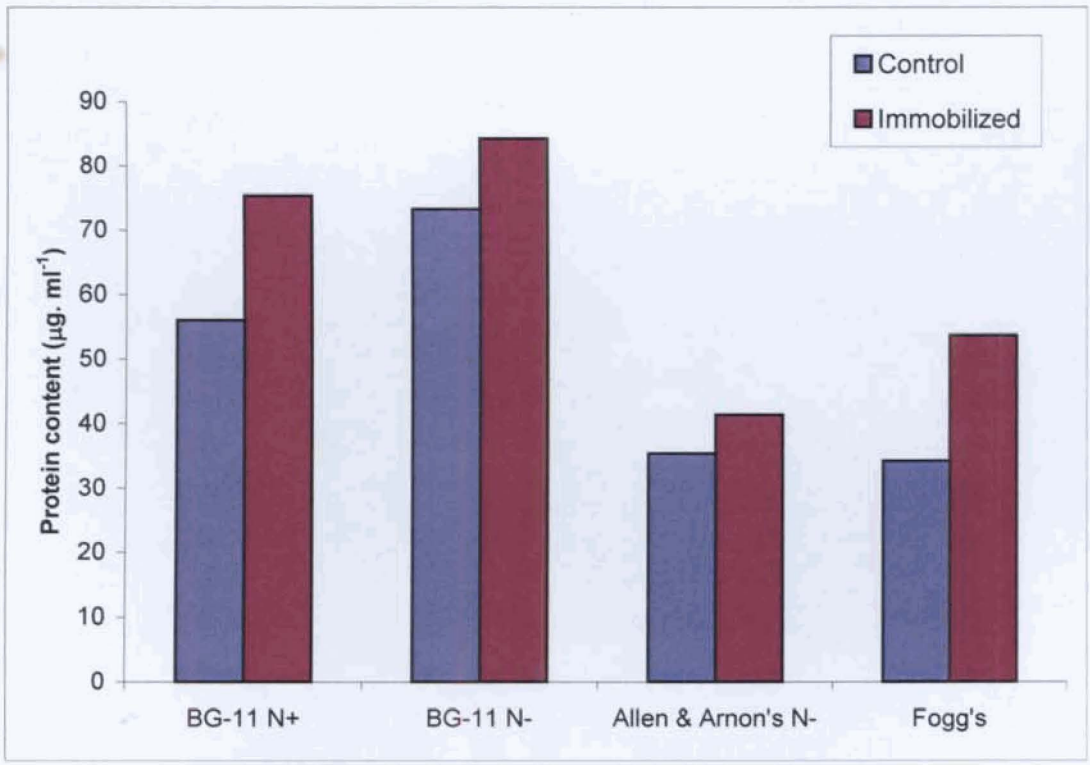


Fig.91 Effect of immobilization on the protein content of *Scytonema cincinnatum* after two months of incubation.

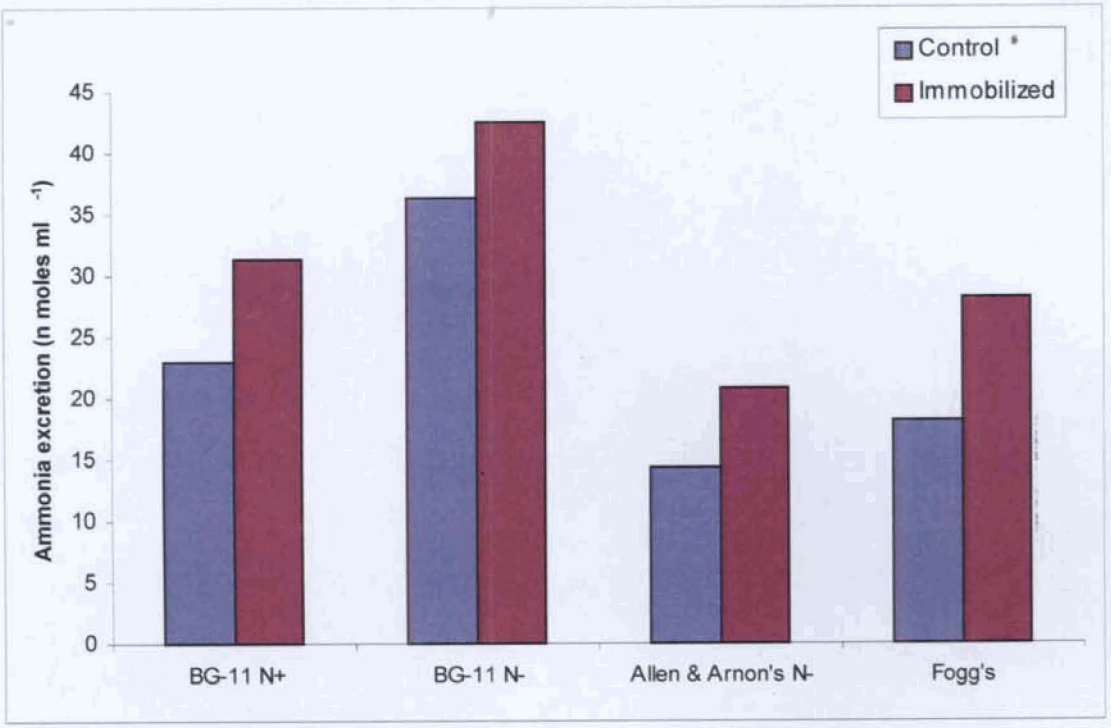


Fig.92 Effect of immobilization on ammonia excretion by *Westiellopsis prolifica* after two months of incubation.

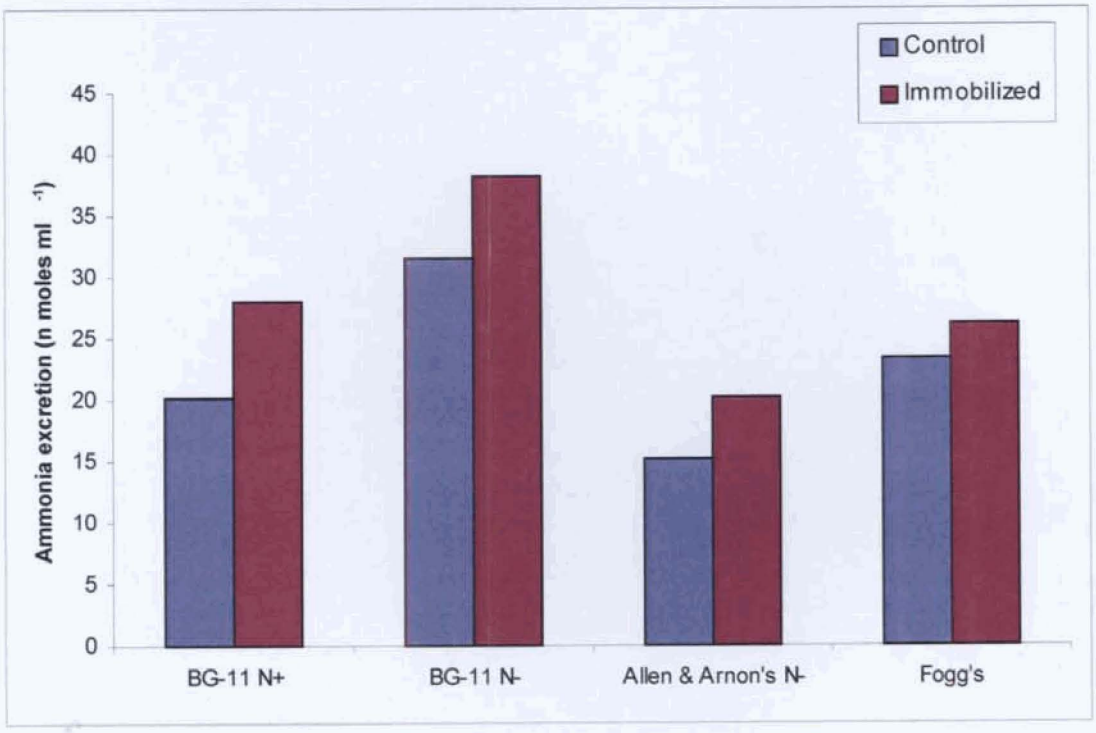
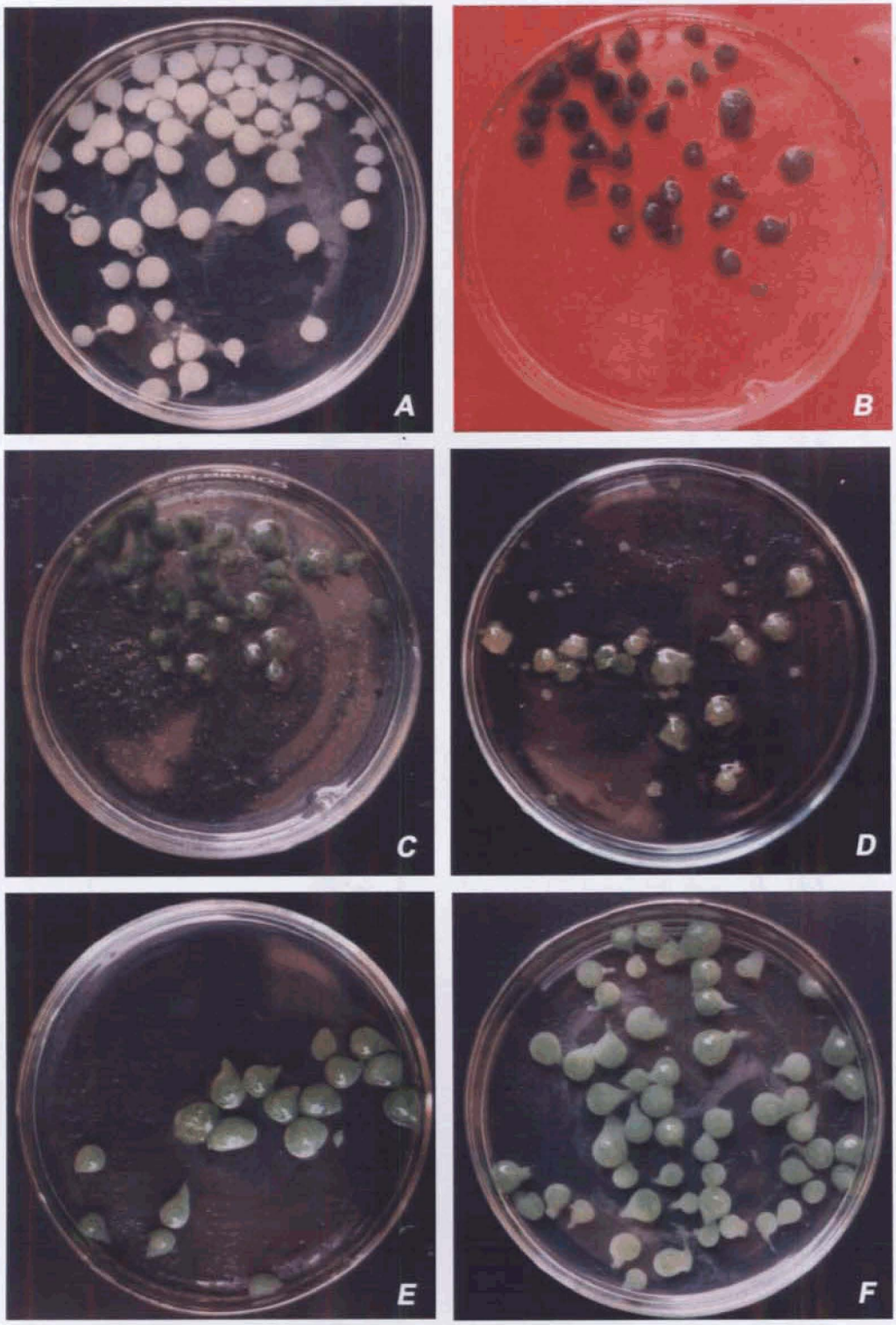


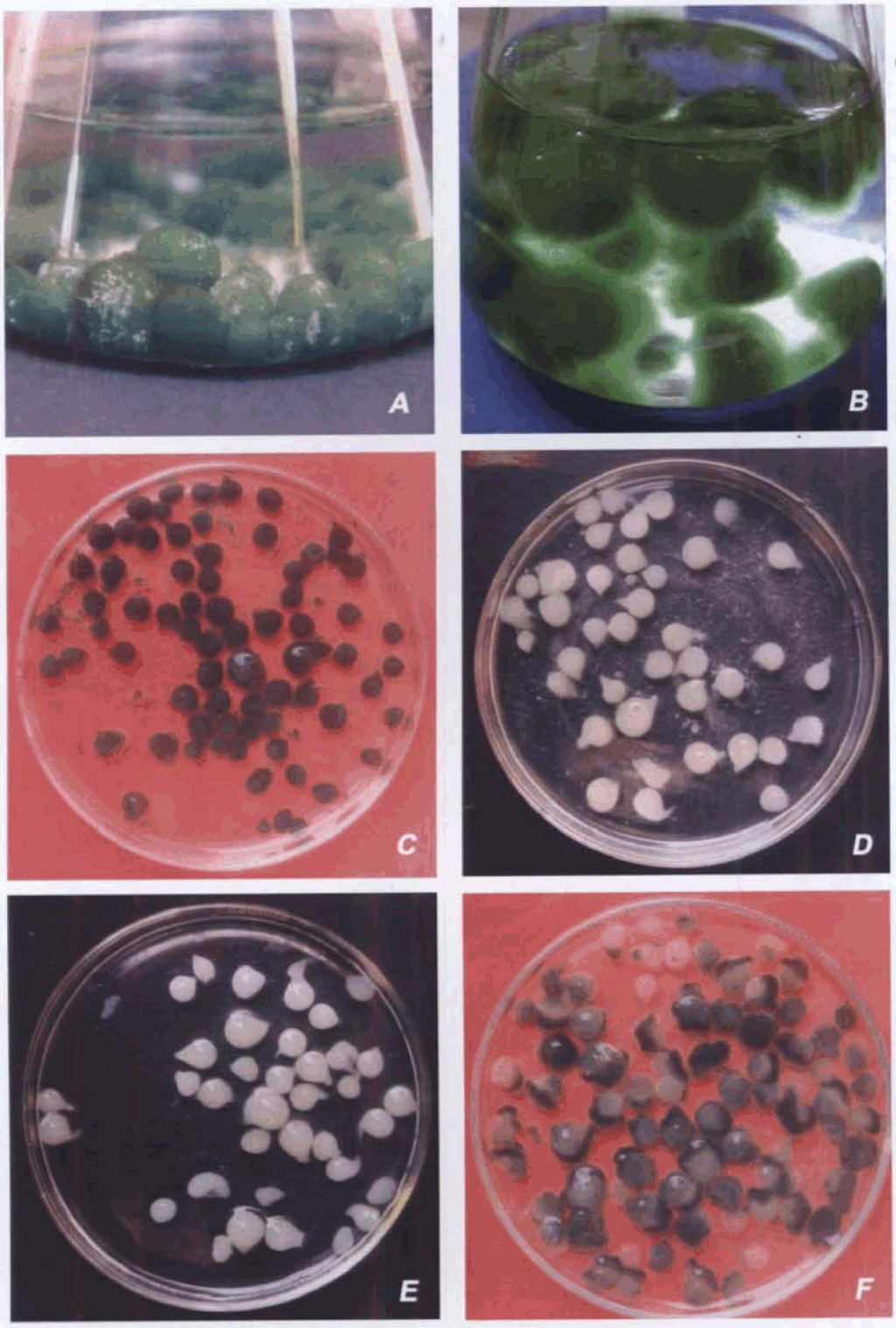
Fig.93 Effect of immobilization on ammonia excretion by *Scytonema cincinnatum* after two months of incubation.

PLATE 4



A. A Mixture of W. prolifica and S. cincinnatum beads at the time of inoculation
B. W. prolifica in BG - 11 N^+ medium after 20 days of growth
C. W. prolifica in BG - 11 N^+ medium after 30 days of growth
D. S. cincinnatum in BG - 11 N^+ medium after 30 days of growth
E. W. prolifica in BG - 11 N^- medium after 20 days of growth
F. S. cincinnatum in BG - 11 N^- medium after 20 days of growth

PLATE 5



A. *W. prolifica in BG - 11 N⁻ medium after 20 days of growth*
B. *W. prolifica in BG - 11 N⁻ medium after six months of growth*
C. *W. prolifica in BG - 11 N⁻ medium after 30 days of growth*
D. *S. cincinnatum in Allen & Arnon's N⁻ medium after 30 days of growth*
E. *W. prolifica in Allen & Arnon's N⁻ medium after 30 days of growth*
F. *W. prolifica in Fogg's medium after 30 days of growth*

4.9.2. Discussion

Nitrogen fixing cyanobacteria are known to contribute nitrogen to the rice plants under natural ecological conditions. These nitrogen fixing cyanobacteria when immobilized in solid matrices such as polyurethane foam, sugarcane waste, calcium or sodium alginate gels, etc. have excreted more ammonia than under free living conditions (Aruna *et al.*, 1998). Biosynthetic capacities of immobilized cyanobacteria (Brouers *et al.*, 1988, Kannaiyan, 1991; Kannaiyan *et al.*, 1992a) have been exploited in recent years. Kannaiyan (1991) has investigated the immobilization of nitrogen fixing symbiotic cyanobiont *Anabaena azollae* in polyvinyl and polyurethane foams for their growth behaviour and ammonia production. Immobilized cyanobacteria have also been utilized to study generation of oxygen and NADPH, fixation of nitrogen and production of hydrogen (Kerby *et al.*, 1983; Muallem *et al.*, 1983) and to study whole cell photosynthesis (Tamponnet *et al.*, 1985).

Immobilization is the process of attaching cells or their constituent biocatalyst to a solid matrix such that the cells do not move independently when placed in a fluid environment. Immobilization can be carried out either by physical means such as adsorption or entrapment in a gel or foam matrix by chemical methods such as covalent binding. The advantages of immobilization are stabilization of catalytic activity, often enhanced reaction rates due to concentration of catalyst surface, ease of seperation and reuse of catalyst from soluble products and process control in a bioreactor

(Brouers and Hall, 1986 and Hall *et al.*, 1998). Immobilized culture system could be used for exploiting biosynthetic potential of the photosynthetic cells. Cells immobilization occurs naturally by the formation of microbial mat as well as by adhesion to available surfaces. The cyanobacterial cultures *Anabaena azollae* and *Anabaena variabilis* when immobilized in polyurethane foam, polyvinyl foam and in calcium alginate beads have been shown to release ammonia extracellularly (Kerby *et al.*, 1986; Brouers *et al.*, 1988, Kannaiyan *et al.*, 1992a,b; 1994). Shi *et al.* (1987) and Aruna *et al.* (1998) have shown that immobilized *Anabaena azollae* in polyvinyl foam, polyurethane foam and alginate beads doubled the nitrogenase activity by increasing heterocyst frequency. Mahesh and Kannaiyan (1992, 1993) observed higher nitrogenase activity in cyanobacterial cultures immobilized in solid matrices than freeliving cultures. They also proposed that the higher nitrogenase activity under immobilized state might possibly due to the colonization and accumulation of higher number of algal cells inside the solid matrices. This can be explained due to the presence of higher cell numbers of cyanobacteria including heterocyst which is responsible for N_2 fixation. Moreover the solid matrices might have provided a favourable situation for colonization of cyanobacteria and for its better adaptation and thereby increased nitrogenase activity.

The present investigation agrees with the above findings. There are reports that the increased ammonia excretion upon immobilization might be due to the changes occurred in the permeability of the cell membrane of

cyanobacteria and subsequent increased activity in metabolites production (Yamamoto *et al.*, 1974). The effects of immobilization in calcium alginate on the morphological and functional parameters of cyanobacteria have been established (Vincenzini *et al.*, 1986). Immobilization enhances the functional and storage longevity of cells (Dainty *et al.*, 1986; Barbotin *et al.*, 1987). The cyanobacteria could colonize and grow well under immobilized state with their cellular and functional properties intact with active vegetative cells and heterocyst. Samal and Kannaiyan (1992) have shown that the increase in the heterocyst number is directly correlated with an increase in nitrogen fixing activity.

It is quite clear from the results that, the immobilization has increased the number of heterocysts in both strains of cyanobacteria and thereby increase the nitrogenase activity. Both *Westiellopsis prolifica* and *Scytonema cincinnatum* show comparatively higher chlorophyll-a and protein contents under immobilized state than under free living conditions. These results are also in accordance with the findings of Paul and Kannaiyan (1998). N_2 fixation and photosynthesis are interrelated process in nature and the pigment constitution and synthesis of protein in *Anabaena azollae* complex could trigger N_2 fixing capacity (Kannaiyan, 1990). Increase in the protein content due to immobilization reflects on its nitrogen contribution to the soil system. Their capacity to release nitrogenous compounds spontaneously substantiates their utilization as efficient biofertilizers in the rice fields. Kannaiyan (1990) has reported the possibility of continuous supply of

ammonia by immobilized *Anabaena azollae* under field conditions, because the immobilized *Anabaena azollae* could continuously supply ammonia to rice plants. Similar results were also reported by Brouers and Hall (1986), Brouers *et al.* (1988) and Kannaiyan *et al.*, (1994). Fay (1992) reported that nitrogenase synthesis inside the heterocysts is regulated by the availability of fixed nitrogen, NH_4^+, NO_3^-, NO_2^- and other forms of combined nitrogen repress the synthesis of the enzyme.

The inoculation of immobilized cyanobacteria alongwith the nitrogen fertilizer has a significant influence on the growth of the crop and ultimately on the grain and straw yields of rice. It increases the growth of rice crop by virtue of their continuous photoproduction of ammonia in flood water ecosystem (Aruna *et al.*, 1998).

In the present investigation an attempt has also been made to examine the effect of different media on the longevity of storage of cyanobacteria entraped in alginate beads. Alginic acid was selected for immobilization of the cyanobacteria because of low cost and simplicity of the method, higher mechanical strength of the beads and free diffusion of the nutrients (Williams and Munnecke, 1981; Smidsord and Skjak-Braek, 1990; Kierstan and Bucke, 2000). Encapsulation provides not only protection, but a more stable microenvironment for the entraped microbial cells. Cyanobacteria entraped in alginate remained stable after a few drying or wetting cycles in soil and it is an effective means of increasing microbial population within confined environment for agricultural and allied

applications. Now-a-days immobilization technique have been used for pollutant degradation by *in situ* bioremediation of chemically contaminated soils (D'Souza, 1999; Sar and D'Souza, 2002). The present investigation suggests that alginate immobilization in BG-11 N^- medium can be used for the preservation of cyanobacteria for more than one year. This results agrees with the findings of Dhar and Vaishya (1995) and Bimal and Pandey (2003). Thus immobilization enhances the biomass content, pigment production, increase in the number of heterocyst, increased ammonia excretion and N_2 fixation, thus it can be efficiently utilized as biofertilizers in rice fields.

4.10. EFFECT OF CYANOBACTERIAL INOCULATION ON THE GROWTH AND YIELD OF RICE

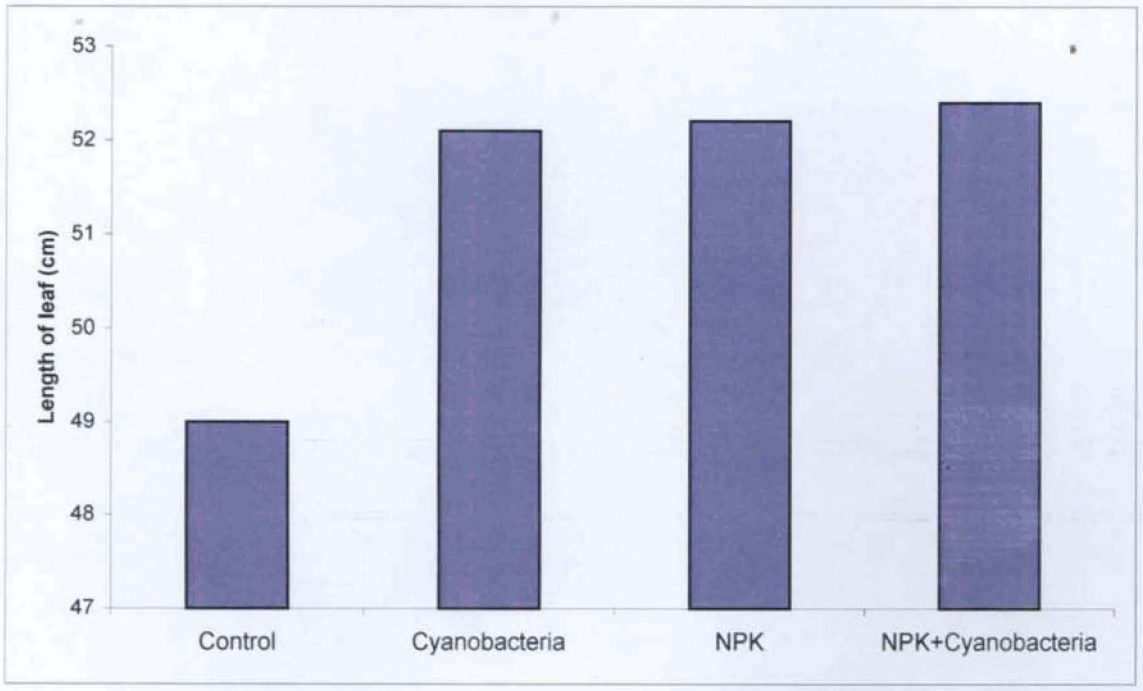
4.10.1. Results

Pot culture experiments was conducted on the growth and yield of paddy (Jaya variety). 2 g dried cyanobacterial mixture of *Westiellopsis prolifica* and *Scytonema cincinnatum* was inoculated into the soil, seven days before transplantation and moisture was maintained to facilitate the cyanobacterial growth. The data (Table 87 & Fig. 94, 95) showed the mean of leaf length, spike length and yield of straw and grain of plants in presence or absence of inoculated cyanobacterial mixture, NPK fertilizer and combination of NPK and cyanobacterial mixture after 120 days of transplantation. The results showed that maximum increase in leaf length, spikelength, straw yield and grain yield was obtained in the pots inoculated

with NPK fertilizer and cyanobacteria and about 60% increase in the grain yield and 69% increase in straw yield over control was obtained in this treatment. There was significant increase in the length of leaves and spike due to treatment with cyanobacteria, NPK fertilizer and combination of NPK + cyanobacteria over the control. The grain and straw yield was increased by 43% and 50% respectively by the application of cyanobacteria over the control.

Table 87. Effect of cyanobacterial inoculation on the growth and yield on Paddy

Treatments	Leaf length (Mean of 25 leaves) (cm)	Spike length (Mean of 15 spikes) (cm)	Straw yield (Average of 5 plants) (g)	Grain yield (Average of 5 plants) (g)
Control	49.0 ± 0.02	21.5 ± 0.01	6.3 ± 0.02	4.0 ± 0.03
Cyanobacteria	52.1± 0.01	23.3 ± 0.01	9.5 ± 0.03	5.8 ± 0.03
NPK	52.2 ± 0.03	24.3± 0.02	10.1 ± 0.02	6.1 ± 0.01
NPK + Cyanobacteria	52.4 ± 0.04	24.9 ± 0.03	10.7 ± 0.01	6.4 ± 0.02



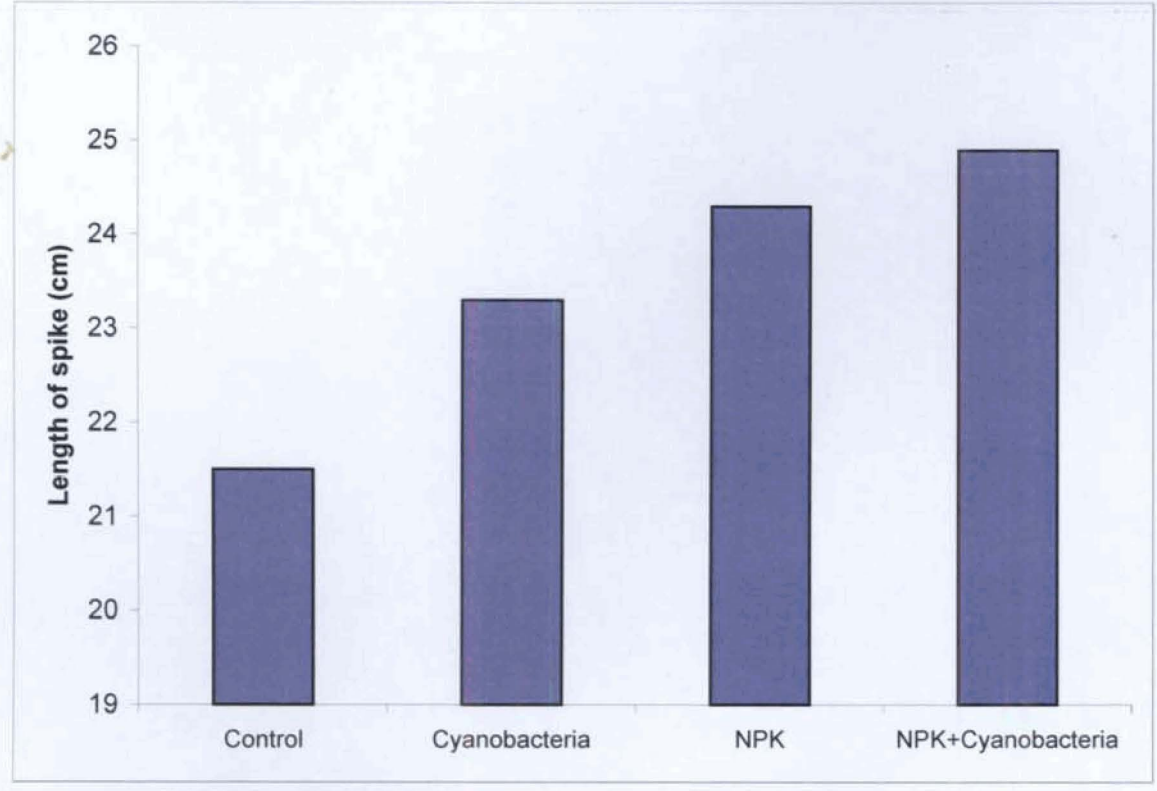
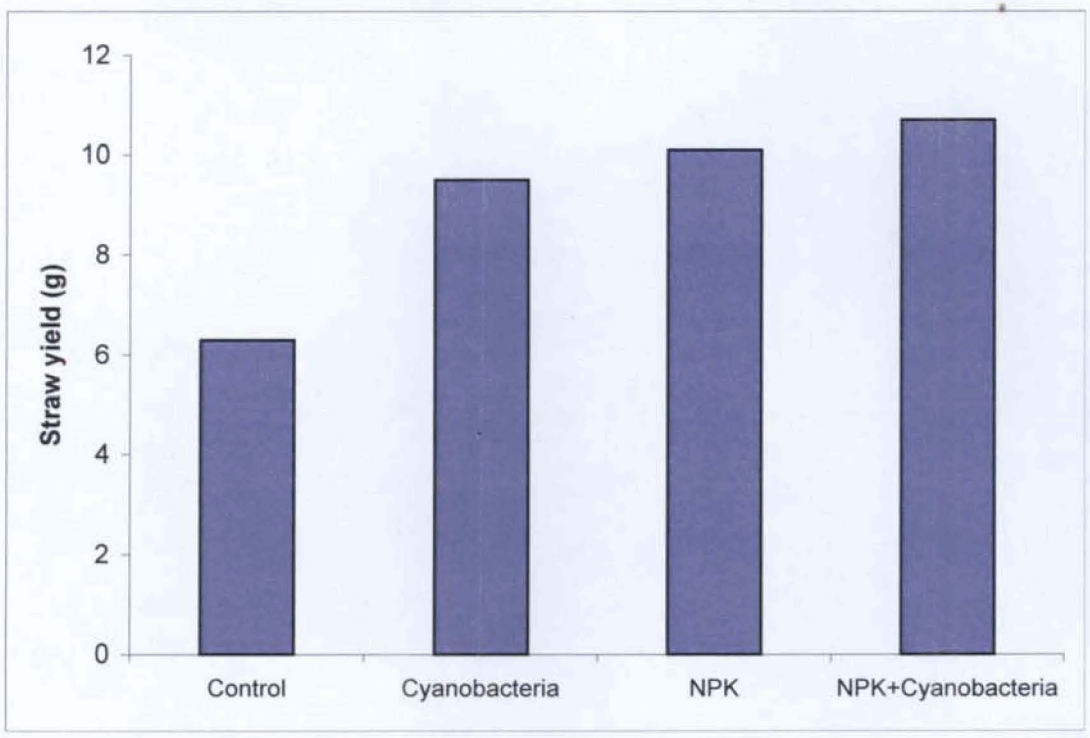


Fig.94 Length of leaf, and length of spike in the experimental pots treated with cyanobacteria, NPK and NPK + cyanbacteria



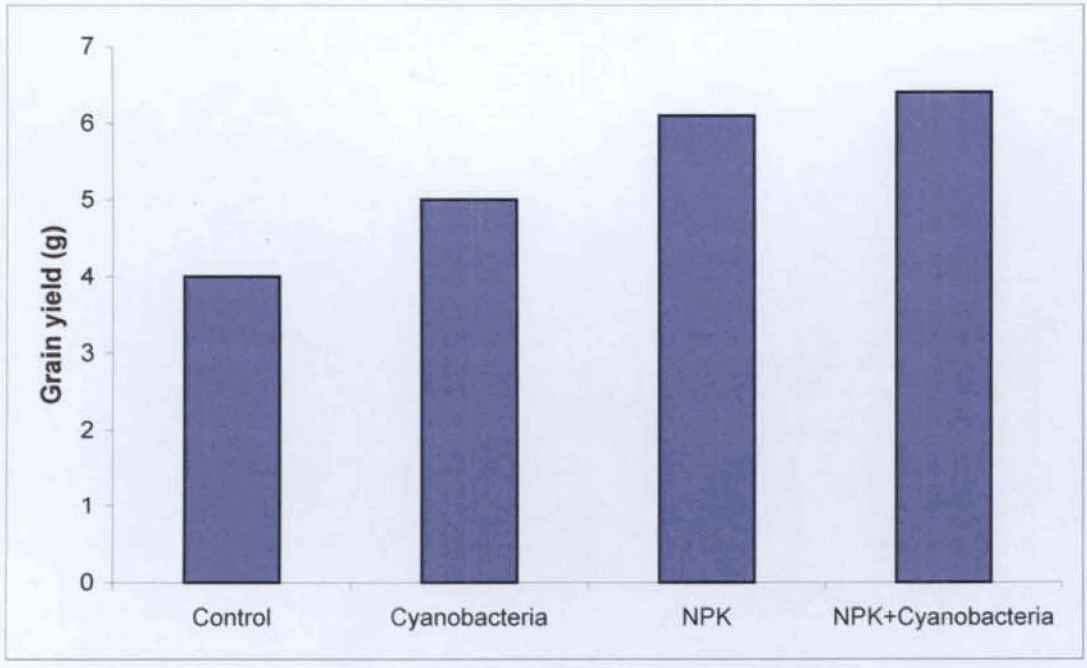


Fig.95 Straw yield and grain yield in the experimental pots treated with cyanobacteria, NPK and NPK + cyanobacteria.

4.10.2. Discussion

Paddy is largely grown in wetland condition with a layer of standing water which encourages the growth of cyanobacteria. The paddy requires a large amount of nitrogen for its growth and grain yield, which is being supplied mainly through chemical fertilizers. The chemical fertilizers deteriorates the fertility of the soil and increase the cost of production. While biofertilizers play an important role in improving the fertility of the soil and thereby reduces the input cost. In this context the cyanobacterial nitrogen fixation is an important contribution to the soil fertility in rice fields. The use of cyanobacteria as biofertilizer for rice cultivation is quite prevelent in many parts of India.

There are evidences about the fixation of elementary nitrogen by cyanobacteria contributing to the nitrogen status of the paddy fields (Bunt, 1961; Goyal and Venkataraman, 1970; Goyal, 1987, 1993; Kolte and Goyal, 1986; Roger and Kulasooriya, 1980; Rather and Mir, 1987; Singh 1961; Sankaram, 1967; Singh and Lakshmi Devi, 1974; Subrahmanyan *et al.*, 1965a,c; Venkataraman, 1966, 1972, 1975, 1981; Watanabe, 1962, 1965, 1978).

Cyanobacteria can also be used along with fertilizer nitrogen to improve the existing crop. This fact has been well established by the study of many earlier workers like Goyal and Venkataraman (1970), Singh (1978), Kannaiyan *et al.* (1982), etc. There are reports that cyanobacteria in addition to fixing atmospheric nitrogen release biologically active compounds

into the soil and these compounds are then assimilated by higher plants significantly enhancing their growth (Venkataraman and Neelakantan, 1967; Dadhich *et al.*, 1969; Metting and Pyne 1986) and yield (Aiyer *et al.*, 1972; Singh *et al.*, 1972; Venkataraman, 1972; Adhikary and Pattnaik, 1991). Cyanobacterial application also increases the height of plants (Singh, 1961; Singh, 1984), leaf length (Watanabe *et al.,* 1951; Watanabe, 1954); number of tillers (Jalapathi Rao *et al.*, 1977); number of spikelets per panicle (Alimagno and Yoshida, 1975; Jalapathi Rao *et al.*, 1977), etc. The water soluble products of eight *Calothrix* spp. and an *Anabaena* sp., had a rhizogenous effect and stimulated plant organs (Koptiyera and Tantisiurenka, 1971). There are reports that the treatment of rice seedlings with cyanobacterial cultures or their extracts attribute to the enhancement of germination and a faster seedling growth due to cyanobacterial exudates (Jacq and Roger, 1977). The probable nature of the substances has been to that of gibberellin (Gupta, 1966; Gupta and Shukla, 1967). Also, the growth pattern of rice seedlings treated with cyanobacterial filtrate from *Aulosira fertilissima* resembled seedlings treated with gibberellic acid (Singh and Trehan, 1973). High frequency germination (91%) was obtained in carrot artificial seeds encapsulated in calcium alginate gel containing 400 mgl^{-1} of extract from a marine cyanobacterium, *Synechococcus* sp. (Wake *et al.*, 1992).

The cyanobacterial inoculation not only increased plant height, grain and straw yield but also improves the soil fertility which increase the crop

growth (Sankaram, 1971; Kaushik, 1985). There are reports that the increased soil fertility is in terms of organic carbon and total nitrogen at flowering and harvest of paddy (Managave *et al.,* 1998; Patil *et al.*, 1998). Cyanobacterial inoculation also improves the protein content of the grains (Tahmida Begum *et al.*, 1990).

Bagal and Patil (1984) reported that cyanobacterial application can reduce the fertilizer nitrogen dose to an extent of 16%. They also observed that the fertilizer treatment in combination with cyanobacteria was significantly superior over other treatments. The increase in yield due to the inoculation of *Tolypothrix* in well drained fields was reported to be 15% while in badly drained fields about 25% (Watanabe *et al.*, 1951). Roger (1991) after 260 field experiments concluded that the cyanobacterial inoculation may enhance the average grain yield by 3q/ha. Singh *et al.* (1992) noted a maximum increase in grain yield up to 6.25 q/ha in different varieties of paddy by cyanobacterial inoculation in ten districts of Uttar Pradesh. Singh *et al.* (1998) found that the application of cyanobacteria in paddy fields by subsequent year increases the concentration of the viable inocula in the field as expected, which further increase the soil fertility in a more economic manner. Further use of cyanobacteria in paddy fields can add something more than biological nitrogen fixation *i.e.*, vitamins, aminoacids and other growth regulating substances which may be beneficial to the crop plants (Fogg, 1939; Venkataraman and Kaushik, 1980; Cheema *et al.*, 1987; Singh *et al.*, 1992). The increase in the subsequent crop might be due to the

release of nitrogen either from extra cellular products of cyanobacteria added or by the decomposition of the latter (Tahmida Begum *et al.*, 1990).

It has been reported that cyanobacteria may contribute 20-30 kg biologically fixed nitrogen per hectre in rice cultivation (Venkataraman, 1972; Goyal, 1982; Sharma and Gupta, 1983) and also improves physical and chemical properties of soil significantly (Aiyer *et al.*, 1971, 1972; Roychoudhury *et al.*, 1979). Singh *et al.* (1998) observed that the application of cyanobacterial biofertilizer along with 75% of the recommended dose of nitrogen fertilizer gives higher yield than that of 100% recommended dose of nitrogen fertilizer and can save 25% nitrogen. According to Goyal and Venkataraman (1970) there is an increase of 34% to 71% in the yield of some high yielding rice varieties was found due to the inoculation of a mixture of *Cylindrospermum musicola, Tolypothrix tenuis, Aulosira fertilissima* and *Nostoc* species.

It is evident from the present study that the application of cyanobacteria had a beneficial effect on the growth and yield of paddy. There are findings that cyanobacteria which inoculated to the rice fields rarely establish due to variations in the ecological factors prevailing there, often quite different from that of its original habitat. This may be the main reason of failure of cyanobacterial inoculation technology. There are reports that foreign strains inoculated in the soil rarely establish (Roger, 1991), which possibly was the reason of not obtaining significant benefits through cyanobacterial inoculation in certain trials (Reddy *et al.*, 1986). In the

present study, the inoculated cyanobacteria form distinct patches on the pots after harvest. It shows that the region specific isolates could adapt well to the local physicochemical conditions and establish well in the experimental pots. Thus for the production of cyanobacterial biofertilizer, use of regional isolates should be preferred. However, results of the present study showed that by inoculating the selected natural species of cyanobacteria isolated from a particular agroclimatic region, the cyanobacterial biofertilizer technology can be substantially improved.

Moreover, since the present agricultural technology is most effective in terms of high levels of production, its full potential can be exploited only with the application of recommended doses of the plant nutrients. For developing countries like India, one of the most limiting factor in crop production today is the chemical fertilizer, which continues to be in short supply and expensive. For these reasons, there is a great deal of world wide interest today in biological nitrogen fixation as a supplement to the use of chemical fertilizers. Nitrogen fertilizer replacement by cyanobacterial application in paddy fields has enormous impact on management and economy of nitrogen fertilizer on one hand and environmental protection including the ecological balance on the other. Since rice is a staple food of more than 60% of the world's population and in India, it is the most important and extensively grown food crop occupying an area of 40 million hectares with an average yield of 17 quintals per hectare, the study on its more economic mode of production is significant.

Summary

5. SUMMARY

Application of microbial inoculants as biofertilizers has became a prospective alternative to abate and ameliorate the ecodeterioration of agro ecosystems. Biological Nitrogen Fixation, a system developed by only microorganisms, is considered to be the "terminus technicus" devised by nature done at ambient temperature and 1 atmospheric pressure. In the present study two diazotrophic cyanobacterial isolates *i.e., Scytonema cincinnatum* and *Westiellopsis prolifica* collected from the rice fields of Kerala were studied to confirm the biofertilizer value of the strains, the growth and various biochemical characters, such as ammonia excretion, production of proteins, concentration of photosynthetic pigments such as chlorophyll, carotenoids and phycobiliproteins were estimated under different parameters was undertook during the present course of investigation. The cyanobacteria were isolated using the standard streak plate and dilution techniques and maintained in the BG-11 medium in the culture room with 25 $\pm$ 1°C temperature and 1500-2000 lux light. The experiments were conducted under the laboratory conditions with both strains of cyanobacteria. The results of the investigation are summarised below:

1. BG-11 N^- medium offers as a better medium for cyanobacterial growth than BG-11 N^+ medium even though they grow in Allen and Arnon's N^- and N^+ and Fogg's medium also.

2. Both cyanobacterial strains recorded considerable growth and biomass generation and the cyanobacterium *Westiellopsis prolifica* registered maximum growth rate than *Scytonema cincinnatum.*

3. The protein and photosynthetic pigments of the cyanobacterial strains showed quantitative variation in composition. *Westiellopsis prolifica* recorded higher protein, chlorophyll, carotenoid and phycobiliprotein content over *Scytonema cincinnatum.*

4. The heterocyst frequency and ability to release ammonia were high in both strains of cyanobacteria while *Westiellopsis prolifica* exhibited higher ammonia excretion.

5. Both strains of cyanobacteria can grow well in wide acidic to alkaline range of pH *i.e.*, from pH 3 to pH 9 but showed its maximum activity at alkaline range. *Scytonema cincinnatum* exhibited its maximum activity at pH 7.4 whereas *Westiellopsis prolifica* exhibited at pH 8.

6. Response of cyanobacterial strains to NaCl showed that *Westiellopsis prolifica* can grow well from 0.1 M to 0.9 M NaCl with maximum activity at 0.6 M NaCl. While *Scytonema cincinnatum* is a non halotolerant form and it can tolerate up to 0.4 M NaCl and after three weeks, the growth was retarded.

7. Response of cyanobacterial strains to exogenous carbon compounds revealed that *Scytonema cincinnatum* prefers mixotrophic growth in which the growth was higher in glucose in light while

chemoheterotrophic growth was completely absent. In *Westiellopsis prolifica* both mixotrophic and chemoheterotrophic growth was noticed in which sucrose was the most preferred substrate than glucose.

8. Response of cyanobacterial strains to carbofuran showed that *Westiellopsis prolifica* can grow well from 10 ppm to 60 ppm carbofuran with maximum growth at 50 ppm carbofuran. While *Scytonema cincinnatum* showed maximum growth at 30 ppm carbofuran and at higher concentrations of carbofuran the green colour of the filament got faded and became yellowish.

9. The effect of neemcake on cyanobacterial strains showed that different strains responded differently to the neem. *Westiellopsis prolifica* is tolerant to neem cake application and stimulated the growth. It can thrive well up to 400 ppm neemcake solution with maximum activity at 300 ppm, whereas *Scytonema cincinnatum* was non tolerant to the neemcake application and the filaments disintegrated at higher concentrations.

10. Response of cyanobacterial strains to the herbicide 2,4-D showed that the cyanobacterium *Westiellopsis prolifica* can thrive well up to 10 ppm 2,4-D with maximum growth at 5 ppm, while *Scytonema cincinnatum* showed maximum growth at 1 ppm, and could not thrive in higher concentrations of 2,4-D.

11. Response of cyanobacterial strains to the organic and inorganic forms of fertilizers showed that cyanobacteria can respond well in these

forms of fertilizers. The ammonia excretion was maximum in the superphosphate grown cyanobacterial cultures than in nitrogen and potassium grown cultures. The growth rate of cyanobacterial strains showed that 20 ppm ammonium sulphate provide better growth than urea. 5 ppm superphosphate promoted maximum growth while the cyanobacterial cultures grown in potassium sulphate at 20 ppm showed better growth than in the cultures grown in commercial potash.

12. Cyanobacterial strains can be easily immobilized in alginate beads and stored in BG-11 N^- medium without losing the viability of cells for more than a year. BG-11 N^+ medium showed signs of bead disintegration after one month of incubation. In Allen and Arnon's N^- medium and in Fogg's medium the growth rate was very slow and Allen and Arnon's N^+ medium showed bead disintegration after one or two days of incubation.

13. The effect of cyanobacterial inoculation on the growth and yield of paddy in pot culture showed that a combination of NPK fertilizers and cyanobacterial strains would be more effective than the application of NPK or cyanobacterial strains alone. A mixture of NPK and cyanobacterial strain induced increased leaf length, spike length, straw yield and grain yield than the single application of NPK and cyanobacterial strains.

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