Effect of pesticides on cyanobacteria used in paddy cultivation

DISSERTATION

Submitted in partial fulfillment for the award of the degree of

MASTER OF SCIENCE

IN BIOTECHNOLOGY

Submitted by

Swati

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Under the Guidance of

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JUNE, 2016

CERTIFICATE

This is to certify that the thesis entitled **"Effect of pesticides on cyanobacteria used in paddy cultivation"** submitted by **Swati (301401014)** in partial fulfillment of the requirement for the award of Degree of **Masters in Science** in Department of Biotechnology, Thapar University, Patiala, is a record of student's own work carried out by her. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

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DECLARATION

I hereby declare that the work which is being presented in this thesis **"Effect of pesticides on cyanobacteria used in paddy cultivation"** submitted by me for the award of the degree of **Masters in Science** in Department of Biotechnology, Thapar University, Patiala, is true and original record of my own independent and original research work carried out under the supervision of **Dr. Dinesh Goyal**. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

Date: 15/07/16

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301401014

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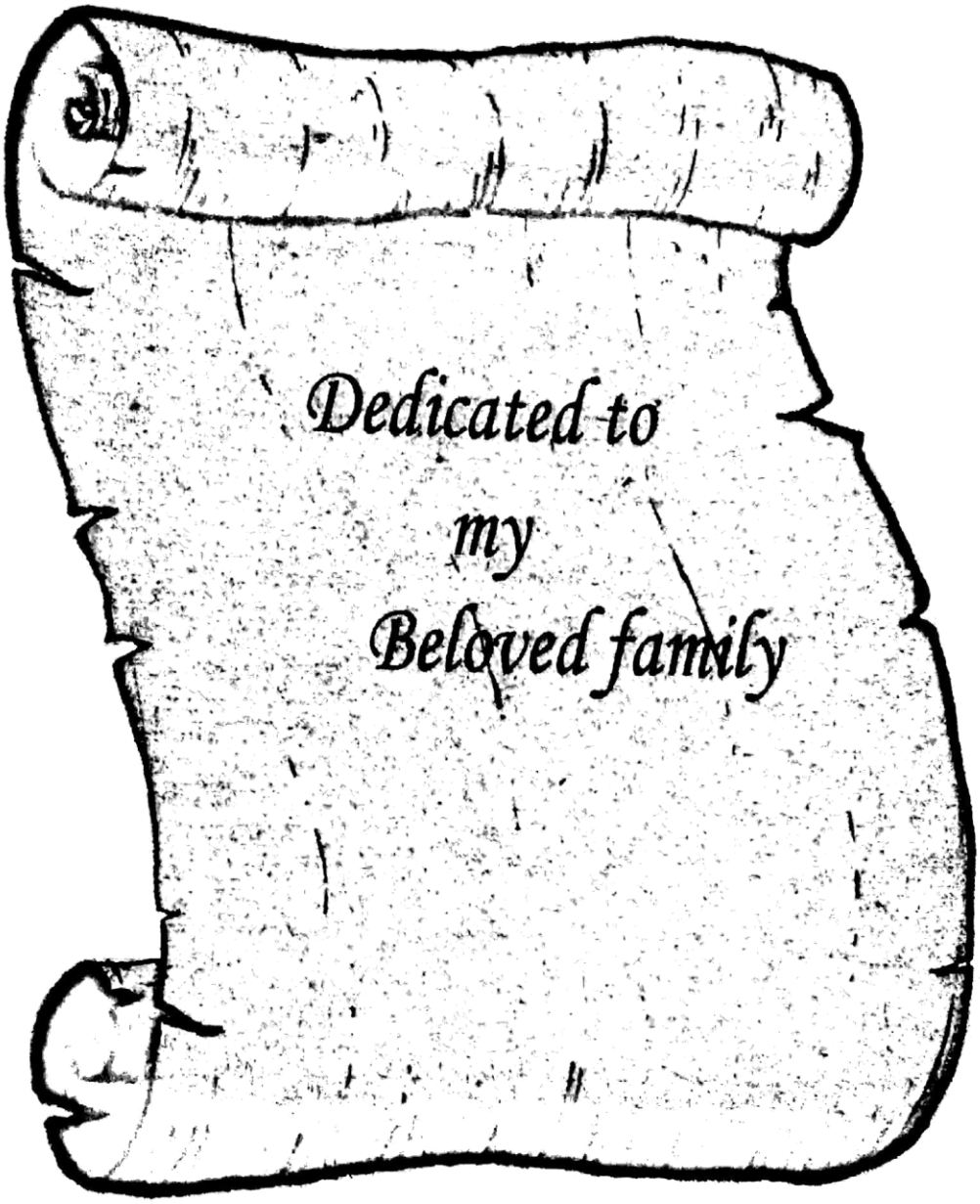
Dedicated to
my
Beloved family

LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
BGA	Blue Green Algae
$CaCl_2$	Calcium Chloride
CAT	Catalase
Chl	Chlorophyll
$Co(NO_3)_2$	Cobalt Nitrate
$CuSO_4$	Copper Sulphate
EDTA	Ethylene Diamine Tetraacetate
et al.	And others
Fe_2SO_4	Ferrous Sulphate
H_2O	Water
H_2SO_4	Sulphuric Acid
H_3BO3	Boric acid
HCl	Hydrochloric acid
K_2HPO_4	Di Potassium phosphate
KH_2PO_4	Potassium dihydrogen phosphate
KOH	Potassium Hydroxide
Mg	Miligram
Mg_2SO_4	Magnesium Sulphate
ml	Mililitre
$MnCl_2$	Manganese Chloride
MoO_3	Molybdenum trioxide
$NaNo_2$	Sodium Nitrite
$NaNO_3$	Sodium Nitrate
NH_3	Ammonia
NH_4^+	Ammonium
NiR	Nitrite Reductase
nm	Nano meter
NR	Nitrate Reductase

POD	Peroxidase
ppm	Parts per million
PS	Photosystem
SOD	Superoxide Dismutase
UV	Ultra violet

LIST OF SYMBOLS

μ	Micro
g	Gram
O_2	Oxygen
N_2	Nitrogen
L	Litre
%	Percentage

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ABSTRACT

Cyanobacteria or Blue Green Algae are ecologically important group of organisms since some forms are capable of fixing atmospheric nitrogen. In present studies effect of different herbicides (pretilachlor and butachlor) and insecticides (monocrotophos and chlorpyrifos) on physiological activity of *Anabaena variabilis* and *Desmonostoc* was analyzed. Tolerance limit, biomass, carbohydrate content, nitrate reductase activity and heterocyst frequency was analyzed in presence of different concentration of pesticides. Lower concentration (0.5 ppm) of pretilachlor and butachlor stimulates the growth of *Anabaena variabilis* and at high concentration (8 ppm) growth was inhibited. In case of chlorpyrifos and monocrotophos, both at low concentration i.e. 0.5 ppm and at higher concentration i.e. 8 ppm the cyanobacterial strain *Anabaena variabilis* there was no inhibition in growth. *Desmonostoc* have no significant effect on the growth in presence of herbicides where as showed positive growth in 8ppm of monocrotophos and chlorpyrifos. *Desmonostoc sp.* is able to tolerate higher concentration of herbicides (butachlor and pretilachlor) and insecticides (monocrotophos and chlorpyrifos) as compared to *Anabaena variabilis*. Since at low concentration of insecticides the physiological process of organism was not adversely affected, the organism can be used in biofertilizer technology program for insecticides (monocrotophos and chloropyrifos) and herbicides (butachlor and pretilachlor) applied fields.

CHAPTER 1 INTRODUCTION

Rice is a staple food for about 50% of world's population that resides in Asia, where 90% of the world's rice is grown and consumed. In India, rice occupies above 44mha of the cultivated area, out of which 20.5 mha are irrigated lands and would need to produce 143 million tonnes of rice to meet the growing demand by 2030 (Subbaiah *et al.*, 2001).

Cyanobacteria (Blue Green Algae) are the major component for nitrogen fixation in paddy cultivation and also for improving yield production (Saadatnia and Riahi, 2009). They constitute the largest, most diverse, and most widely distributed group of photosynthetic prokaryotes (Stanier and Cohen-Bazire, 1971), which play important role in maintaining soil fertility. In the soil, they are reported to improve organic content, water holding capacity, nitrogen status, and release vitamins, plant stimulating hormones, extra cellular polysaccharides and also solubilize phosphates. More than half of the total nitrogen used in paddy crop derives from the native soil nitrogen pool which is maintained through biological nitrogen fixation by both heterotrophs and autotrophs in soil (Singh *et al.*, 2013). Use of cyanobacterial biofertilizer is considered to be a good management of paddy fields since their use not only increases fertility of the soil but is also eco-friendly.

Pesticides are commonly used in rice field to control weeds, pests and other kind of diseases to improve crop yield. The term pesticide is combination of variety of different types of chemicals including herbicides, insecticides, fungicides, and rodenticides, among others (National Pesticide Information). So, the utilization of cyanobacterial biofertilizer in paddy fields requires that strains be tolerant or able to degrade a variety of routinely used agrochemicals, including herbicides. Therefore, studying the effect of pesticides on cyanobacteria, their tolerance and their use as biofertilizers may play an important role in the establishment of soil fertility.

A large number of herbicides such as anilofos, butachlor, pendimethalin, pyrazolsulfuron ethyl, oxadiargyl and pretilachlor are recommended for weed control in the rice fields. Indiscriminate use of herbicides is a great threat to environment and beneficial soil microflora (Singh *et al.*, 2015). Herbicides cause deleterious effects on cyanobacteria by influencing their growth, photosynthesis, nitrogen fixation, biochemical composition and metabolic activities (Eladel *et al.*, 2010).

Present study was aimed at investigating the effect of some commonly used pesticides in paddy cultivation on the growth of *Anabaena variabilis* and *Desmonostoc* sp. that are used as biofertilizer in rice cultivation.

CHAPTER 2 REVIEW OF LITERATURE

Rice (*Orzya sativa*) is used as staple food in all over the world and it serve as a staple food source for more than half of the world population (Xu *et al.,* 2016). Use of high-yielding crop varieties, fertilization, and pesticides has contributed substantially to the tremendous increases in food production over the past 50 years. Use of pesticides in agriculture control pests, diseases, weeds and other plant pathogens and eliminate yield loss and maintain high produce quality (Fuad *et al*., 2012).

2.1 Cyanobacteria

Cyanobacteria are phototrophic autotrophs are a group of Gram negative bacteria. Filamentous and heterocystous forms of cyanobacteria fix atmospheric nitrogen and enrich the soil with nitrogen and humus. They improve organic content, water holding capacity, nitrogen status and plant stimulating hormones, extra cellular polysaccharides and also solubilizes phosphate. It is reported that more than half of the total nitrogen used in paddy crops derived from the native soil nitrogen pool which is maintained through biological nitrogen fixation by both heterotrophs and autotrophs in soil. Cyanobacteria have been applied in rice fields as biofertilizers for improving crop yield (Stainier, 1971).

Paddy soils have a natural population of cyanobacteria (Blue-Green Algae), which are source of nitrogen fixation. Cyanobacteria take ammonia by passive diffusion or as ammonium (NH_4^+) a specific uptake system (Mishra and Pabbi, 2004). Biological nitrogen fixation is the most common nitrogen fixation process, estimated 193 x 10^6 tons of nitrogen is fixed through biological nitrogen fixation each year all over the world (Factsheet 39; Cornell University). Addition of biofertilizer in rice field can increases the yield up to 25% (Chaudhary and Kennedy, 2004). Different kinds of cyanobacteria are found in paddy fields some are heterocystous and some are non heterocystous (Table 1) which help in nitrogen fixation.

Table 1: Cyanobacteria found in Paddy Field (Desikachary, 1959 and Kushwaha, 2015)

Heterocystous		Non heterocystous
Anabaena aequalis	*Cylindrospermum musicola*	*Pseudanabaena*
Anabaena constricta	*Cylindrospermum sphaerica*	*Limnothrix*
Anabaena fertilissima	*Nostoc muscorum*	*Jaaginema*
Anabaena laxa	*Nostoc calcicola*	*Geitlerinema*
Anabaena oryzae	*Scytonema hofmanni*	*Spirulina*
Anabaena volzii	*Scytonema iyengari*	*Leibleinia*
Anabaenopsis arnoldii	*Calothrix castellii*	*Schizothrix*
Cylindrospermum majus	*Calothrix fusca*	*Arthrospira*
Cylindrospermum indicum	*Calothrix gloeocola*	*Planktothrix*
Gloeotrichia raciborskii	*Calothrix linearis gardneri*	*Pseudophor midium*
Homoeothrix hansgirgi	*Calothrix simplex*	*Phormidium*
Homoeothrix varians	*Calothrix tenella*	*Porphyrosiphon*

The factors affecting the growth and nitrogen fixing capacity are poorly understood. A varied factors like soil reaction, salinity, nutrients availability, predators, grazers, pesticides and climatic factors play a great role in the activity and growth of

cyanobacteria in soil. In this investigation attempts have been made to understand the effect of currently utilized pesticides in paddy field.

2.2 Pesticides and their classification

The term pesticide includes variety of different types of chemicals that acts as herbicides, insecticides, fungicides, and rodenticides, among others (National Pesticide Information). The pesticides are classified (Fig.1) depending upon target species and mode of action (Table 2). According to the application the main classes of pesticides include herbicides (that kills weeds), fungicides (that kills fungi) and insecticides (that kills insects). The major classification is based on the chemical nature i.e. organochlorine, organophosphorous, thiocarbamates, pyrethroids and neonicotinoids (Tiwana *et al*., 2007).

Organochlorines

An organochloride, organochlorine, chlorocarbon, chlorinated hydrocarbon, or Chlorinated solvent is an organic compound containing at least one covalently bonded chlorine atom. Organochlorine pesticides are insecticides composed primarily of carbon, hydrogen and chlorine. Most of them break down slowly and can remain in the environment long after application and in organisms long after exposure. Some of the examples of organocholrine pesticides include alpha-BHC, beta-BHC, delta-BHC, gamma-BHC (Lindane), heptachlor, endosulfan, methoxychlor, aroclor and dichlorodiphenyltrichloroethane (Tiwana *et al*., 2007).

Organophosphates

Organophosphates bind to acetylcholinesterase and other cholinesterases leading to distruption of nerve impulses, killing the insects or inability to carry out normal function. organophosphorus compounds includes acephate, azinphos-methyl, bensulide, chlorethoxyfos, chlorpyrifos, diazinon, dicrotophos, dimethoate, disulfoton, ethoprop, fenamiphos, fenitrothion, fenthion, fosthiazate, methamidophos, monocrotophos and malathion (Tiwana *et al*., 2007).

Carbamates

Carbamate is derived from carbamic acid, closely related to organoposphorus compound in mode of action and resistance development. Bendiocarb, carbofuran, carbaryl, dioxacarb, fenobucarb, fenoxycarb, isoprocarb and methomyl are some of the examples of carbamate insecticides (Tiwana *et al*., 2007).

Pyrethroids

These are group of pesticides which are applied against hoysehold pests. These are nonpersistent, which is a sodium channel modulators, and are much less acutely toxic than organophosphates and carbamates Cypermethrin,cyfluthrin, deltamethrin, etofenprox, fenvalerate, permethrin, phenothrin, prallethrin, resmethrin and tetramethrin are some examples of the pyrethroids (Tiwana *et al*., 2007).

Neonicotinoids

These are synthetic analogues of natural compound nicotine. They are applied as sprays, drenches, seed and soil treatments, often as substitutes for organophosphates and carbamates. Treated insects exhibit leg tremors, rapid wing motion, stylet withdrawal (aphids), disoriented movement, paralysis and death. Acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam are some of neonicotiniods (Tiwana *et al*., 2007).

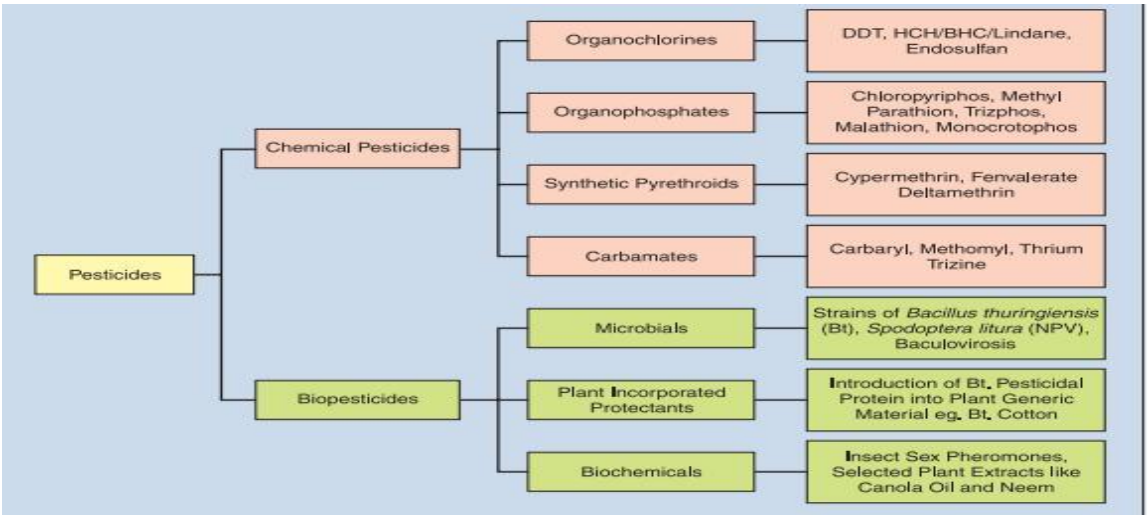


Fig 1: Classification of pesticides (Tiwana *et al*, 2007)

Different pesticides have different effect on cyanobacteria and their site of mechanism of action is different (Table 2).

Table 2: Mechanism of action of pesticides (Delorenzo *et al.,* 2000)

Pesticide class	Groups included	General toxic effect	Specific site of action
Herbicides	Urea, cyclic urea, triazines, aclayinilides, phenyl carbamates, triazinones	Photosynthesis inhibition	Hill reaction of electron transport.
	Bipyridiniums	Photosynthesis inhibition (light reaction)	Reducing side of photosystem I
	Pyridazinones	Biosynthesis inhibition	Carotene accumulation
	Chloroacetamide	Biosynthesis inhibition	Fatty acid synthesis
	Dinitroanilines, phosphoric amides, chlorthaldimethyl, propyzamide, cholchicine, terbutol	Biosynthesis inhibition	Microtubule formation
Broad-spectrum biocides	Chlorophenols	Multiple inhibiting actions	Phophosphorylation, protein synthesis, lipid biosynthesis
	Tributyl tins, trialkyl tins	Respiratory system inhibition	Mitochonrial ATPase

Central Insecticides Board & Registration Committee had given a list of insecticides (Table 3), herbicides (Table 4) and their dosage value, which can be used in paddy fields.

Table 3: Insecticide commonly used in paddy field

Insecticide	Common name of the pest	Dosage (gm / ha)
Acetamiprid	BPH	10-20
Azadirachtin 0.03%	Leaf roller, Stem borer, BPH	0.03
Azadirachtin 0.15%	Thrips, Stem borer, Brown Plant hopper, Leaf folder	10
Benfuracarb 3%	Stem borer, Leaf folder, BPH	1000
Bifenthrin 10%	Stem borer, leaf folder & Green leaf hopper	50
Bromadiolone	Field Rat, Large Bandicota Indian house rat	0.005
Buprofezin	BPH, GLH,WBPH	200
Carbaryl 5%	Leaf roller/folder, Brown plant hopper	1250
Carbaryl 10%	Blue Jassid, Case worm	2500
Carbaryl 50%	Brown Plant Hopper, Stem Borer	1000
Carbofuran 3%	Brown plant hopper Gall midge, Stem borer, GLH, Nematodes	750
Carbosulfan 25%	Green leaf hopper, White plant hopper ,Brown plant hopper	200-250
Cartap hydrochloride 4%	Stem borer, Leaf folder, Whorl Maggot	750-1000
Cartap hydrochloride 50%	Stem borer, Leaf folder	500
Chlorantraniliprole	Stem borer and leaf folder	
Chlorpyrifos	Stem borer, Leaf Roller, Gall midge	1000

(Source: Central Insecticides Board & Registration Committee MAJOR USES OF PESTICIDES (Registered under the Insecticides Act, 1968) up to 31.08.2015)

Table 4: Herbicide commonly used in paddy field

Herbicide	Weed species	Dosage kg/ ha
Anilofos 30%	*Echinochloa crusgalli, Echinochloa colonum, Cyperus difformis , Cyperus iria, Eclipta alba, Ischaemum rugosum, Fimbristylis sp., Marsilea quadrifoliata*	0.3-0.45
Anilofos 18%	*Echinochloa crusgalli, Echinochloa colonum, Cyperus difformis, Cyperus iria, Eclipta alba, Ischaemum rugosum, Fimbristylis sp.*	0.30-0.45
Bensulfuron Methyl 60%	*Marsilea quadrifoliata, Eclipta alba, Ammania baccifera, Ludwigia parviflora ,Sphenoclea Zeylenica , Monochoria vaginalis , Alternanthera sessillis Cyperus iria , Cyperus difformis , Fimbristylis miliacea, Scirpus royeli*	0.06
Bispyribac Sodium 10%	*Ischaemum rugosum, Cyperus difformis, Cyperus iria ,*	0.02
Butachlor 50 %	*Echinochloa colonum, Echinochloa crusgalli, Cyperus difformis, Cyperus iria , Eclipta alba, Fimbristylis miliacea, Ludwigia parviflora, Sphenoclea zeylanica, Monochoria vaginalis*	1.25-1.5
Carfentrazone ethyl 40%	*Chenopodium album , Melilotus Indica , Melilotus alba, Medicago denticulata, Lathyrus aphaca, Analgalis rvensis , Vicia sativa, Circium arvense , Rumex sp, Malwa sp.*	0.02
Cinmethylin 10%	*Cyperus iria, Fimbristylis milacea, Monochoria vaginalis, Commelina Benghalensis, Echinocloa crusgalli, Marsilea minuta*	0.75- 1
Clomazone 50%	*Echinochloa crusgalli, Echinochloa colonum, Cyperus difformis, Cyperus iria, Ludwigia parviflora, Eclipta alba*	0.4 - 0.5
Metsulfuron Methyl 20%	*Cyperus rotundus, Spheanochlea spp., Fimbristylis sp. , Ludwigia parviflora, Marsilea quadrifoliata*	0.04
Pretilachlor 37%	*Echinochloa crusgalli, Echinochloa colonum, Cyperus difformis , Cyperus iria , Digitaria sanguinalis, Fimbristylis miliacae, Eclipta alba, Ludwigia parviflora, Monochoria vaginalis*	0.60-0.75

(Source: Central Insecticides Board & Registration Committee MAJOR USES OF PESTICIDES (Registered under the Insecticides Act, 1968) up to 31.08.2015)

Pesticides recommended by Punjab Agricultural University, Ludhiana

These are the 18 pesticides (Table 5) recommended by the Punjab Agriculture University to be used in paddy field (Bhushan *et al*, 2013).

Table 5: Pesticides recommended by Punjab Agriculture University, Ludhiana

Herbicide
Anilofos
Pendimethelene
Pretilachlor
Sethoxydim
Bensulfuron
Metsulfuron Methyl
Thiobendcarb
Oxadiargyl
Ethoxysulfuron
Methyl Butachlor

Insecticide
Quinalphos
Carbendazim
Endosulfan
Carbendazim
Chlorpyrifos
Monocrotophos

Fungicide
Carboxin
Carboxin
Copper Oxychloride
Pencycuron
Propiconazole

2.3 Effect of herbicide on cyanobacteria

The use of pesticides forms an important component of present day improved agricultural technology. Singh *et al* in 2015 studied the effect of herbicide (pretilachlor) on cyanobacterium *Desmonostoc muscorum* PUPCCC 405.10, results in decreased nitrate, nitrite uptake reduced, nitrogen assimilation activity and heterocyst formation (Table 6).

Effect of pretilachlor and UV-B on *Azolla* species was studied by Prasad *et al*., 2015. Biomass accumulation, photosynthetic pigments; chlorophyll a, b and carotenoids, photosynthetic oxygen yield and photosynthetic electron transport activities i.e. photosystem II (PS II) and photosystem I (PS I) in both the species declined with the increasing doses of pretilachlor and UV-B radiation.

Natural tolerance of some rice field diazotrophic cyanobacteria on exposure to herbicide Bispyribac-sodium was studied by Tariya and Dubey, (2014). Herbicides can result in stimulatory effect at lower concentration and at higher concentration result in inhibitory effect on plant as cyanobacteria has many metabolic mechanisms similar to that of higher plants.

Singh and Khattar, (2013) studied the effect of anilofos on cyanobacterium *Synechocystis sp*. strain PUPCCC 64. The organism tolerated up to 25 mg/L, it caused inhibitory effect on chlorophyll a, carotenoids, phycocyanin, allophycocyanin and phycoerythrin and superoxide dismutase, catalase and peroxide activity increased (Table 6).

Singh and Khattar, (2012) studied the effect of aniflos on photosynthesis, respiration, nitrogen assimilation, and antioxidant system in a diazotrophic rice field cyanobacterium *Anabaena torulosa.* Photosyntesis, nitrogen assimilation, nitrate and ammonium uptake, nitrogenase and glutamine synthetase activity decreased and superoxide dismutase, catalase, and peroxidase activity increased.

Deng *et al,* (2012) studied the effect of herbicides on growth and hydrocarbon content in *B. braunii.* Diuron was the most toxic than diquant, metachlor and bensulfuron-methyl strongly inhibited algal growth. Biomass yield decreased to 30.6% of control, but hydrocarbon content increased from 34.9 to 42.4% of dry biomass in the presence of a 0.1 mg/L concentration of diuron after 30 days of exposure. Diquat, S-metolachlor and bensulfuron-methyl had strong inhibition of algal growth, and inhibition increased with increasing concentration. Diquat at 5mg/L inhibited the growth of *B. braunii*, but there is hydrocarbon content to 43.3% of dry biomass when treated with 5 mg/L diquat. S-metalochlor had significant effect on both algal growth and hydrocarbon content. Bensulfuron-methyl had no significantly effect on hydrocarbon content. Fluridone at 0.1 mg/L did not impact algal growth, but it decreased hydrocarbon content from 34.9 to 13.2%. Thiobencarb at 1 mg/L inhibited algal growth slightly and decreased hydrocarbon content from 34.9 to 7.8%. Dinoterb at 0.1 mg/L had a significant effect on both algal biomass and hydro-carbon content.

Okmen and Ugur, (2011) studied the influence of bispyribac sodium on nitrogenase activity and growth of cyanobacteria isolated from paddy fields. In *Anabaena sp. strains O-X2, O-4* and *O-8* it were found that very low concentrations of bispyribac-sodium (5 μg/ml) stimulated both nitrogenase activity and growth but increased concentrations repressed both nitrogenase activity and growth (Table 6).

Effect of two photosynthetic inhibitor herbicides, atrazine (both purified and formulated) and DCMU, on the growth, macromolecular contents, heterocyst frequency, photosynthetic O_2 evolution and dark O_2 uptake of wild type and multiple herbicide resistant (MHR) strain of diazotrophic cyanobacterium *A. variabilis* was studied and both the pesticides showed gradual decrease in the growth but DCMU was more toxic to the growth of cyanobacteria even at lower concentrations (Singh *et al.,* 2011).

The effects of molinate on the activities of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and glutathione S-transferase were evaluated in *Nostoc muscorum*, a freshwater cyanobacterium (Galhano *et al.*, 2011). Herbicide concentration increased lipid peroxidation, electrolyte leakage and increase in production of proline.

Effects of three herbicides often used in rice field, viz. propanil, pretilachlor and glyphosate, on the performance traits of Anabaena fertilissima (Inderjit and Kaushik, 2010). Chlorophyll content was slightly affected of pretilachlor, glyohosate but propanil decreases chlorophyll content (Table 6).

Alteration in growth and physiological activities in *Chlorella vulgaris* under the effect of photosynthetic inhibitor diuron was studied by Fayez, (2007). The growth of *C. vulgaris* towards increasing concentrations of diuron in terms of cell number and dry weight was significantly decreased. The cell number at 0.1 μm and 0.5 μm diuron decreased by 2.4 and 6.7 fold and the dry weight was lowered by 2.5 and 5.5 fold, w.r.t control Photosynthetic pigments were significantly decreased in response to increasing diuron treatments.

Chen *et al*, (2007) examined the effects of butachlor, bensulfuron-methyl, and dimethoate on the growth, photosynthesis, and photoinhibition of the edible cyanobacterium (*Nostoc*) and studied the productivity reduction and use of pesticides. Inhibition of growth was observed in the presence of very high concentrations of bensulfuron-methyl and dimethoate. Mild stimulation of photosynthesis was observed at low concentrations of these three pesticides (Table 6).

Table 6: Effect of herbicide on cyanobacteria

Pesticide	***Algae***	**Factor affected**	**Result**	**References**
Pretilachlor 2.5-10 ppm	*Desmonosto cmuscorum*	Nitrate uptake Nitrite uptake Ammonia uptake Nitrogen fixation	With increase in pesticide concentration nitrate uptake, nitrite uptake, ammonium uptake and nitrogen fixation decreased by 73 %, 68%, 0.70 µmol/mg, and 40% respectively.	Singh *et al*., 2015
Pretilachlor 5-20 µg/ml	*Azolla sp.*	Biomass Photosynthetic pigments	With increase in concentration of pesticide biomass and photosynthetic pigment decreases.	Parsad *et al*., 2015
Nomnigold 75-300 ppm	*Anabaena circinalis* *Scytonema hofmanni*	Growth Chlorophyll content Nitrogen content	With increase in pesticide concentration, decrease in Chlorophyll content and Nitrogen content was observed	Tariya and Dubey, 2014
Anilofos 5-20 mg/ml	*Synechocystis sp.*	Chlorophyll carotenoid phycocyanin Allophycocyanin phycoerythrin Antioxidant enzymes	Decrease in chlorophyll, carotenoid, phycocyanin, Allophycocyanin and phycoerythrin by 60 %, 89%, 96%, 855, 79% respectively. SOD, POD and CAT activities were stimulated by 180%, 174%, 131% respectively.	Singh *et al*., 2013
Molinate	*Nostoc muscorum*	Proline content Lipid peroxidation	Increased production of proline at higher molinate concentrations (the values rose above control by 45%, 95% and 156% with 0.75, 1.5 & 2mM, respectively. Increased lipid peroxidation by 5.4%, 19% and 28% with 0.75, 1.5 and 2mM of molinate, respectively.	Galhano *et al.*,2011

Pretilachlor 0-80 mg/L And Glyphosate 0-80mg/L	*Anabaena fertilissima*	Biomass Chlorophyll	Lower concentration of pretilachlor enhances biomass; at higher concentration i.e. 40 mg/L inhibits growth. At 10 mg/L enchaces biomass and at 80 mg/L inhibits growth. Chlorophyll content enhanced at 10 ppm and was decreased at 80 ppm.	Inderjit And Kaushik, 2010
Duron 0-0.5 µM	*C.vulgaris*	Biomass Chlorophyll content Nitrate reductase activity	Decrease in biomass, chlorophyll content and Nitrate reductase activity was observed.	Fayez, 2007
Butachlor Bensulfuron-methyl Dimethoate	*Nostoc*	Growth Photosynthesis	Inhibition of growth and photosynthesis at higher concentration of pesticide.	Chen *et al*., 2007

2.4 Effect of Insecticide on cyanobacteria

The effect of lambda cyhalothrin insecticide effect was studied on *Calothrix sp*., higher concentration of this insecticide showed decrease in dry weight Biomass, chlorophyll-a, carotenoids, phycocyanin, and nitrogen contents but it had insignificant effect at lower concentrations (Gupta and Baruah, 2015).

Singh *et al,* (2014) studied the effect of Cartrap Hydrochloride on the non-heterocystous *Leptolyngbya foveolarum*. Lower concentration of Cartrap promoted the growth with high weight of dry biomass, total protien content, photosynthetic pigments, photosynthesis and respiration and at higher concentration inhibited the growth.

Kumar *et al*, (2014) studied the effect of Chlorpyrifos on freshwater cyanobacteria *Chroococcus turgidus NTMS12*. At higher concentration of pesticide, there was increased concentration of proline content, superoxide dismutase activity was increased, and polyunsaturated fatty acids content was decreased (Table 7).

Effect of four commonly used pesticides viz. Bagalol, Mancozeb(fungicides), Thiodan and Phorate (insecticides) was studied on growth and different enzymes of four cyanobacterial species viz. *Nostoc ellipsosporum, Scytonema simplex, Tolypothrix tenuis,* and *Westiellopsis prolifica,* both insecticide and pesticide inhibited the nitrogenase and glutamine synthetase and isocitrate dehydrogenase activity. One anabolic enzyme isocitrate lyase activity was induced (Debnath *et al.*, 2012).

Effect of carbaryl on the cellular changes of *Calothrix brevissima* was given by Habib *et al.*, 2012. They studied that at higher concentration polyunsaturated fatty acids were decreased by 32% and membrane leakage was increased by 27%. The sulfur contatining metabolites cysteine, cystine, and methionine were also increased. The enzymatic and nonenzymatic antioxidants namely glutathione S-transferase, glutathione reductase, reduced glutathione, and oxidized glutathione were also increased shown in table 7.

Effect of different 2, 4 -dichlorophenoxyacetic acid (2, 4-D) concentrations on growth rate, content of protein and chlorophyll-a in *Chlorella vulgaris* and *Spirulina platensis* cells was studied. Herbicidal effect of high concentrations of 2, 4-D. *S. platensis* was found to be more sensitive than *C. vulgaris* and low concentrations have stimulatory effect and higher concentrations have in hibitory effect (Saygideger and Okkay, 2008).

The cyanobacterium *Phormidium valderianum* BDU 20041 is able to grow in the presence of chlorpyrifos (O, O-diethyl-O-[3, 5, 6-trichloro-2-pyridyl] phosphorothioate), a phosphorothioate insecticide. High concentration increased the activity of pesticide-metabolizing enzymes, such as polyphenol oxidase, catalase, superoxide dismutase, esterase, and glutathione S-transferase. Increased activity of catalase and superoxide dismutase provoked the state of oxidative stress, concurrently this circuitously proving the triggered mode of reactive oxygen species mediated degradation of Chlorpyrifos (Palanisami, 2009).

Some metabolic activities in the green alga *Scenedesmus bijuga* had been affected by the insecticide trichlorfon (Fathi, 2003). Reduced the total protein content of in comparison to control values, the cell number and Chl. a content of decreased with increase in trichlorfon concentration.

Table 7: Effect of Insecticide on cyanobacteria

Pesticide	***Algae***	**Factor affected**	**Result**	**References**
Cyhalothrin	*Calothrix*	Biomass , chlorophyll, carotenoids, phycocyanin, and nitrogen contents	Higher concentration has inhibitory effect and lower concentration have no significant effect.	Gupta and Baruah, 2015
Pentachloro phenol	*Microcystis aeruginosa Chlorella vulgaris*	Biomass	Higher concentration has inhibitory effect on growth.	Moaris *et al.,* 2014
Carbaryl	*Calothrix bervissimia*	Polyunsaturated fatty acids Amino acids Enzymatic activites	Higher concentration of carbaryl decreased polyunsaturated fatty acids by 32%. Membrane leakage increased by 27 %. Enzymatic activities decreased.	Habib *et al*., 2012
Chlorpyrifos	*Chroococcus turgidus*	Proline Content SOD & CAD Polyunsaturated fatty acids	Proline and oxidative enzyme content increased and polyunsaturated fatty acid decreased	Kumar *et al*., 2014

Bagalol, Mancozeb Thiodan Phorate	*Nostoc ellipsosporum, Scytonema simplex, Tolypothrix tenuis, and Westiellopsis prolifica,*	Nitrogenase Synthetase Glutamine Synthetase Isocitrate dehydrogenase Isocitrate lyase	Nitrogenase Synthetase, Glutamine Synthetase, Isocitrate dehydrogenase activity inhibited. Isocitrate lyase activity induced	Debnath *et al*., 2012.
2,4-D	*Chlorella vulgaris Spirulina platensis*	Growth rate Protein content Chlorophyll	At higher concentration inhibitory effect and at lower concentration stimulatory effect	Saygideger and Okkay, 2008
Chlorpyrifos Phosphorothioate	*Phormidium valderiaum*	Polyephenol oxidase Catalase Superoxide dismutase Esterase Glutathione S-transferase	Increase in activity of enzyme in presence of higher concentration of pesticide.	Palanisami , 2009
Trichlorfon	*Scenedesmus bijuga*	Protein content Chlorophyll content Cell number	High concentration of trichlorofon decreased the protein, chlorophyll and cell no.	Fathi, 2003

Cyanobacterial cultures *Anabaena variabilis* (ARM 441) and *Desmonostoc sp.*(Isolate C) were grown in BG-11 (-N) medium at 28±2ºC and 2500-3000 lux light intensity for 21 days with 16:8 light/dark cycle. Algal cultures were grown in the test tubes with 5 ml of BG-11 containing different concentration (0.5, 1, 2, 4 and 8 ppm) of herbicides (pretilachlor and butachlor) and insecticide (monocrotophos and chlorpyrifos) for 21 days. There after growth was estimated in terms of biomass and best concentrations and than grown in 300 ml of BG-11 containing 1, 2, 5 ppm butachlor, pretilchlor and 1, 4, 8 ppm monocrotophos, chlorpyrifos. At interval of 7 days growth was estimated in terms of biomass, chlorophyll and physiological activities in terms of nitrate reductase and total carbohydrate content. Heterocyst was also checked microscopically. All experiment was set up in triplicates w.r.t to control (control means without any concentration of pesticide).

Fig.2: Cyanobacterial growth in test tubes and flasks

Composition of BG-11 medium (Stanier *et al.,* 1971)

Constituent's	**g/L**
Sodium nitrate	1.5
K_2HPO_4	0.040
$MgSO_4.7H_2O$	0.075
$CaCl_2.H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA	0.001
Sodium carbonate	0.20
Trace metal mix	1 ml
Distilled water	1000 ml

Trace metal mix composition

Constituent's	**g/L**
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.39
$CO(NO_3).6H_2O$	0.079
$CuSO_4.5H_2O$	0.0494
Distilled water	1000 ml

Cyanobacterial strains used

***Anabaena variabilis* (ARM-441)** a *nitrogen* fixing filamentous, hetrocystous alga.

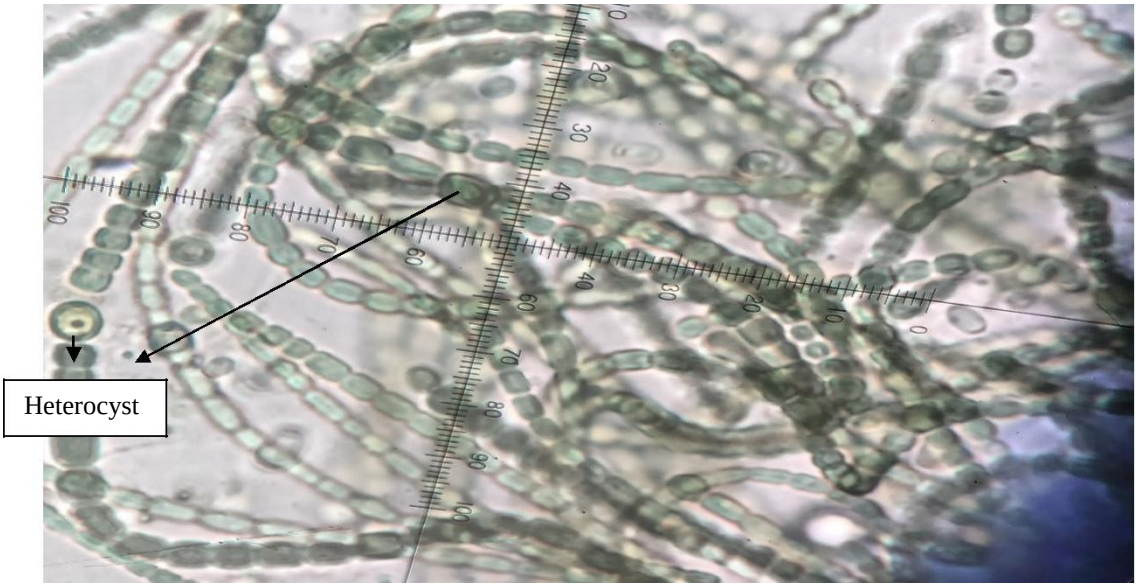


Fig.3: *Anabaena variabilis* ARM 441

***Desmonostoc sp.* (Isolate C)** a filamentous, heterocystous nitrogen fixing alga.

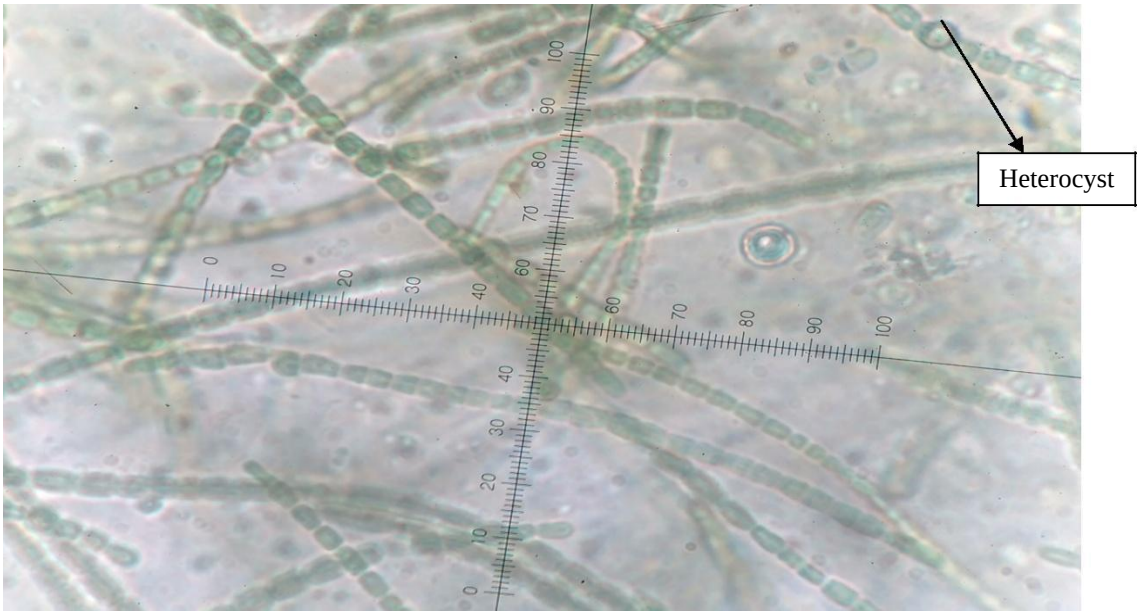


Fig.4*: Desmonostoc* sp. (Isolate C)

3.1 Growth studies

Growth of *Anabaena* sp. and *Desmonostoc* sp. was studied by dry biomass estimation and chlorophyll estimation. Cyanobacterial species were grown in the different concentration of pesticides in 300 ml of BG- 11 medium and after interval of 7 days 100 ml sample was taken for estimation of different parameters.

3.1.1 Dry Biomass Estimation

Biomass was estimated by using method given by Richmond and Gobbelaar, (1986).

Procedure:

1. The whatman filter was soaked in water to saturate and dried overnight at 60^{o}C.
2. The weight of dried filter paper was noted as the initial reading.
3. Took some beads and autoclave them.
4. The cultures were homogenized by vigorous shaking by adding dried beads to it and 10 ml of culture was taken and filtered through previously dried paper using vacuum filtration assembly. Note the wet weight.
5. This was kept for drying and transferred to hot air oven maintained at about 60^{o}C, till constant weight was recorded at room temperature.
6. The whatman filter was soaked in water to saturate and dried overnight at 60^{o}C.
7. The weight of dried filter paper was noted as the initial reading.
8. Took some beads and autoclave them.
9. The cultures were homogenized by vigorous shaking by adding dried beads to it and 10 ml of culture was taken and filtered through previously dried paper using vacuum filtration assembly. Note the wet weight.
10. This was kept for drying and transferred to hot air oven maintained at about 60^{o}C, till constant weight was recorded at room temperature.

Calculations:

Dry biomass = wet weight of algae – dry weight of paper.

3.1.2 Chlorophyll Estimation

Chlorophyll was estimated by acetone method given by Holm, (1964).

Reagents: 80% acetone was prepared by adding 80 ml of acetone and 20 ml of distilled water.

Procedure:

1. 10 ml of algal culture was centrifuged at 10000 rpm for 15 minutes and pellet was washed twice with water.
2. Pellet was incubated overnight in 5 ml of 80% acetone at room temperature.
3. Again centrifuged at 10000 rpm and supernatant was taken.
4. Pigment of solution was analyzed using spectrophotometer by comparing sample of unknown transmission against blank (80% acetone) of 100% transmission at 645 and 660 nm.

Calculations:

$$\text{Chlorophyll} = \frac{(9.78 \times A_{660}) - (0.99 \times A_{645}) \times \text{volume of acetone}}{\text{extract Dry weight Biomass}}$$

3.2 Microscopic determination of heterocyst frequency of filamentous cyanobacteria

Heterocyst frequency was determined method given by Ramirez, (2011).

Procedure:

1.Fix the material in iodine solution.

2.Observed it at 100X magnification using emersion oil under the microscope. 3.Heterocyst cell as well as vegetative cells was counted.

Calculation:

$$\text{Heterocyst frequency (\%)} = \frac{\text{Total no. of Heterocyst}}{\text{Total no. of cell}} \times 100$$

3.3 Physiological Studies

Physiology of *Anabaena sp.* and *Desmonostoc* sp. was studied by nitrate reductase activity and total carbohydrate content. Cyanobacterial species were grown in the different concentration of pesticides in 300 ml of BG- 11 medium and after interval of 7 days 100 ml sample was taken for estimation of different parameters.

3.3.1 Nitrate Reductase activity

Nitrate reductase activity was studied by Lowe and Evans, (1964) method.

Reagents:

A.1% sulphanilamide in 100 ml of 1N HCl.(1g of sulphanilamide in 100 ml of 1N HCl)

B.0.2% NEED (N-1- napthyl ethylene diamine dichloride) [(0.2 g NEED in 100 ml of distilled water)]

Basal Medium Composition

Constituent's	g/L
$NaNO_3$	25
Calcium chloride	2.5
Magnesium sulphate	7.5
K_2HPO_4	7.5
KH_2PO_4	17.5
NaCl	2.5
EDTA	50
$FeSO_4$	4.98
KOH	31
H_2SO_4	1 ml
H_3BO_3	11.43
Trace element mix	1 ml

Trace Element Mix

Constituent's	g/L
$MnCl_2$	1.44
$ZnSO_4$	8.82
MoO_3	0.71
$CuSO_4$	1.57
$CoNO_3$	0.49

Procedure:

1. 10 ml of algal culture was spun at 10000 rpm for 15 minutes and pellet was washed with distilled water twice.
2. Pellet was incubated in 5ml of basal medium at room temperature overnight.
3. 1ml of basal medium was taken from samples and 2ml of sulphanilamide was added and kept at room temperature for 15 minutes.
4. After 15 minutes add 2ml of NEED, and pink color was observed.
5. Absorbance was recorded at 540 nm and the values were calibrated against curve using $NaNO_2$. The NR activity was expressed in terms of µ mole NO_2/ml.

3.3.2 Carbohydrate Estimation

Total carbohydrate content was estimated by Solorzano, (1969) method.

Reagent:

Anthrone Reagent: 200 mg anthrone in 98% of H_2SO_4.

Procedure:

1. 10 ml of algal culture was spun at 10000 rpm for 15 minutes and pellet was dried overnight.
2. 1ml of water was added to dried pellet and 4 ml of anthrone reagent was added.
3. Absorbance was taken at 690 nm and value was calibrated against standard curve of glucose.

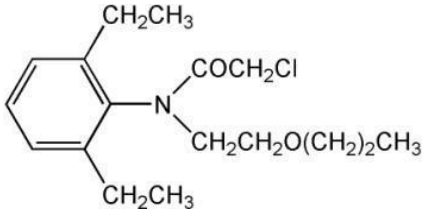
3.4 Pesticides used in present study

Most commonly used insecticides monocrotophos and chlorpyrifos and herbicide pretilachlor and butachlor as identified by small market survey in Patiala and Nabha were used to study their effect on the growth of nitrogen fixing cyanobacteria such as

Anabaena variabilis (ARM 441) and *Desmonostoc* (Isolate C) under flask culture conditions.

Herbicides used

Pretilachlor (organochlorine) (pubchem.ncbi.nlm.nih.gov)

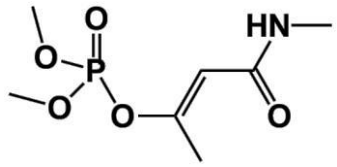
Molecular Formula	C17H26ClNO2
Structural Formula	
IUPAC Name	2-chloro-N-(2,6-diethylphenyl)-N - (2-propoxyethyl) acetamide
Molecular weight	311.84684 g/mol
Physical State	Yellow color Liquid

Butachlor (organochlorine)

Molecular Formula	$C_{17}H_{26}ClNO_2$
Structural Formula	
IUPAC Name	N-(Butoxymethyl)-2-chloro-N-(2,6-diethylphenyl)acetamide
Molecular Weight	311.85 g/mol
Physical State	Yellow color Liquid

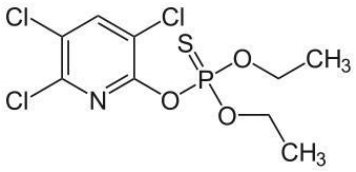
Insecticide used

Monocrotophos (Organophosphate)

Molecular Formula	$C_7H_{14}NO_5P$
Structural Formula	
IUPAC Name	3-Hydroxy-N-methylcrotonamide dimethylphosphate
Molecular Weight	223.2 g/mol
Physical State	Reddish Brown Liquid

(Source: pubchem.ncbi.nlm.nih.gov)

Chlorpyrifos (organophosphate)

Molecular Formula	$C_9H_{11}Cl_3NO_3PS$
Structural Formula	
IUPAC Name	O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate
Molecular Weight	350.9 g/mol
Physical state	colorless oily liquid

Pretilachlor, butachlor are two commonly used herbicides and monocrotophos, chlorpyrifos are two commonly used insecticides these were purchased from the local market under different brand names. These pesticides are not pure; they are mixed with some solvents and other compounds. So, the concentration of pesticides is calculated according to its active ingredient.

Table 8: Details of pesticides used

Pesticides	**Brand name**	**Active ingredient (%)**	**Stock concentration (ppm)**	**Recommended Dose**
Pretilachlor (Herbicide)	Uttam utpad, Chambal fertilizers and Chemicals Ltd	50	500,000	0.75 ppm
Butachlor (Herbicide)	Uttam utpad, Chambal fertilizers and Chemicals Ltd	50	500,000	4.8 ppm
Monocrotophos (Insecticide)	Phoskil, united phosphorus Ltd.	53	530,000	0.2 ppm
Chlorpyrifos (Insecticide)	Chlorguard, Gharda chemical Ltd.	21.5	2,15000	3 ppm

Calculations:

Active ingredient = 50%

i.e. 50 g in 100 ml or 500 mg per ml

1 ppm = 1000mg/ml

So, concentration of stock is 500*1000 = 500,000 ppm

Further dilutions were made from this stock solution.

CHAPTER 4 RESULTS AND DISCUSSION

Pesticide application in paddy cultivation can be acutely toxic to cyanobacteria, which is used as biofertilizer and promotes plant growth by fixing atmospheric nitrogen. In the present study, effect of two herbicides (pretilachlor and butachlor) and two insecticides (monocrotophos and chlorpyrifos) on cyanobacteria was studied. The selection of pesticides was done on the basis of their current use in Patiala and Nabha region. The cyanobacteria used in the study were *Anabaena vrariabilis* (ARM-441) and *Desmonostoc sp*. (Isolate C). *Anabaena variabilis* (ARM-441) and *Desmonostoc sp*. were grown in the presence of different concentrations of pretilachlor, butachlor, monocrotophos and chlorpyrifos (0.5- 8 ppm).

Table: 9 Growth of *Desmonostoc* (Isolate C) in graded concentration of herbicides (Pretilachlor and Butachlor)

Days	Control	Pretilachlor (ppm)					Butachlor (ppm)				
		0.5	1	2	4	8	0.5	1	2	4	8
1	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
6	++	+	+	+	+	+	++	+	+	+	+
7	++	+++	++	+	+	+	+++	+	+	+	+
8	++	+++	++	+	+	+	+++	+	+	+	+
9	++	+++	+++	+	+	+	+++	+	+	+	+
10	++	+++	+++	+	+	+	+++	+	+	+	+
11	++	+++	+++	+	+	+	+++	+	+	+	+
12	++	+++	+++	+	+	+	+++	+	+	+	+
13	++	+++	+++	+	+	+	+++	+	+	+	+
14	++	+++	+++	+	+	+	+++	+	+	+	+
15	++	+++	+++	+	+	+	+++	+	+	+	+
16	++	+++	+++	+	+	+	+++	+	+	+	+
17	++	+++	+++	+	+	+	+++	+	+	+	+
18	++	+++	+++	+	+	+	+++	+	+	+	+
19	++	+++	+++	+	+	+	+++	+	+	+	+
20	+++	+++	+++	+	+	+	+++	+	+	+	+
21	+++	+++	+++	+	+	+	+++	+	+	+	+

+ growth, ++ moderate growth, +++ best growth

Effect of pesticide on growth of cyanobacteria varied with respect to pesticide concentration, pesticide type and the type of cyanobacteria. *Desmonostoc sp*. showed no inhibition in growth at a concentration of 8 ppm of pretilachlor, butachlor, monocrotophos and chlorpyrifos where as, results varied for *Anabaena variabilis.* It showed positive growth at lower concentration (0.5 ppm) of pesticide and inhibited at high concentration (8 ppm).

Table 10: Growth of *Desmonostoc sp.* in graded concentration of Monocrotophos and Chlorpyrifos

Days	Control	Chlorpyrifos (ppm)						Monocrotophos (ppm)					
		0.03	0.5	1	2	4	8	0.03	0.5	1	2	4	8
1	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+
3	++	+	+	+	+	+	+	+	+	+	+	+	+
4	++	+	+	+	+	+	+	+	+	+	+	+	+
5	++	+	+	+	+	+	+	+	+	+	+	+	+
6	++	+	+	+	+	+	+	+	+	+	+	+	+
7	++	+	+	+	+	+	+	+	+	+	+	+	+
8	++	+	+	+	+	+	+	+	+	+	+	+	+
9	++	+	+	+	+	+	++	+	+	+	+	+	++
10	++	+	+	+	+	+	++	+	+	+	+	+	++
11	++	+	+	+	+	+	++	+	+	+	+	+	++
12	+++	+	+	+	+	+	++	+	+	+	+	+	++
13	+++	+	+	+	+	+	++	+	+	+	+	+	++
14	+++	+	+	+	+	+	++	+	+	+	+	+	++
15	+++	+	+	+	+	+	++	+	+	+	+	+	++
16	+++	+	+	+	+	+	++	+	+	+	+	+	++
17	+++	+	+	+	+	+	++	+	+	+	+	+	++
18	+++	+	+	+	+	+	++	+	+	+	+	+	++
19	+++	+	+	+	+	+	++	+	+	+	+	+	++
20	+++	+	+	+	+	+	++	+	+	+	+	+	++
21	+++	+	+	+	+	+	++	+	+	+	+	+	++

+ growth, ++ moderate growth, +++ best growth

Low concentration of monocrotophos and chlorpyrifos supported slightly higher growth compared to untreated control cultures, while at higher concentration growth was inhibited (Table 10). The same results were reported for *Leptolyngbya foveolarum* at 80 ppm concentration of cartap hydrochloride by Singh *et al.*, 2014. Other reports also confirmed the inhibitory effects of insecticides on *Synechocystis* sp. (Singh *et al.*, 2011), *Tolypothrix scytonemoides* (Rajendran, 2006), *Nostoc* (Chen *et al.*, 2007) and

Phormidium valderianum (Palanisami, 2009). Adverse effect of herbicide Anilofos was studied on *Synechocystis* sp. strain PUPCCC 64, where it caused the cell lysis, releasing pigments in the medium and leaving the culture colorless.

Cyanobacterial strain *Desmonostoc* (Isolate C) which showed positive growth in 8ppm of monocrotophos and chlorpyrifos was chosen for present study (Table 10). Lower concentration of insecticides showed positive growth but at higher concentration i.e. 8ppm they showed the maximum growth, even greater than the control, i.e. higher concentration of monocrotophos and chlorpyrifos (8ppm) stimulated the growth of cyanobacteria.

Table 11: Growth of *Anabaena variabilis* (ARM 441) in graded concentration of pretilachlor and butachlor

Days	Control	Pretilachlor (ppm)					Butachlor (ppm)				
		0.5	1	2	4	8	0.5	1	2	4	8
1	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+	+	+
4	+	+	+	+	+	-	+	+	+	+	-
5	+	+	+	+	+	-	+	+	+	+	-
6	++	+	+	+	+	-	++	+	+	+	--
7	++	+++	++	+	+	-	+++	+	+	+	--
8	++	+++	++	+	+	-	+++	+	+	+	--
9	++	+++	+++	+	+	-	+++	+	+	+	--
10	++	+++	+++	+	+	-	+++	+	+	+	--
11	++	+++	+++	+	+	--	+++	+	+	+	--
12	++	+++	+++	+	+	--	+++	+	+	+	--
13	++	+++	+++	+	+	--	+++	++	+	+	--
14	++	+++	+++	+	+	--	+++	++	+	+	--
15	++	+++	+++	+	+	--	+++	++	+	+	--
16	++	+++	+++	+	+	--	+++	++	+	+	--
17	++	+++	+++	+	+	--	+++	++	+	+	--
18	++	+++	+++	+	+	--	+++	++	+	+	--
19	++	+++	+++	+	+	--	+++	++	+	+	--
20	+++	+++	+++	+	+	--	+++	++	+	+	--
21	+++	+++	+++	+	+	--	+++	++	+	+	--

+ growth, ++ moderate growth, +++ best growth, - very low growth, -- no growth

Cyanobacterial strain *Anabaena variabilis* (ARM-441) showed positive growth at 8 ppm of butachlor and pretilachlor (Table 11). Low concentration of butachlor and pretilachlor supported slightly higher growth compared to untreated control cultures, while at higher concentration growth was inhibited. The probable reason could be the breakdown of the pesticide, resulting in uptake of nitrogen present in the compound. Similar results were reported by Singh *et al.*, 2015 for *Nostoc* sp. KK26 at 10 ppm of pretilachlor which stimulated the growth. Higher concentartion of pretilachlor inhibited the growth of *Azolla sp.*, at 20 ppm there was no growth (Parsad *et al*., 2015).

Table 12: Growth of *Anabaena variabilis* (ARM-441) in graded concentration of monocrotophos and chlorpyrifos

Days	Control	Chlorpyrifos (ppm)						Monocrotophos (ppm)					
		0.03	0.5	1	2	4	8	0.03	0.5	1	2	4	8
1	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+
3	++	+	+	+	+	+	+	+	+	+	+	+	+
4	++	++	++	++	++	+	+	+	++	++	++	+	+
5	++	++	++	++	++	+	+	+	++	++	++	+	+
6	++	++	+++	+++	++	+	+	+	+++	+++	++	+	+
7	++	++	+++	+++	++	+	+	+	+++	+++	+++	+	+
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20	+++	++	+++	+++	++	+	++	++	+++	+++	+++	+	++
21	+++	++	+++	+++	++	+	++	++	+++	+++	+++	+	++

+ growth, ++ moderate growth, +++ best growth

In case of chlorpyrifos and monocrotophos, both at low concentration i.e. 0.5 ppm and at higher concentration i.e. 8 ppm the cyanobacterial strain *Anabaena variabilis* there was no inhibition in growth.

The effect of two herbicide and two insecticides on cyanobacterial growth was studied in terms of biomass, chlorophyll content, Nitrate Reductase activity (NR) and heterocyst frequency.

4.1 End point growth curve of *Desmonostoc* (Isolate C) and *Anabaenavariabilis*ARM-441 in graded concentration of pesticides

Overall growth of *Anabaena variabilis* (ARM-441) and *Desmonostoc sp.* (Isolate C) in presence of pretilachlor and butachlor (0.5, 1, 2, 4, 8 ppm) after 21 days of incubation was estimated in terms of dry biomass production (Fig. 5). Cyanobacterial strain

Desmonostoc sp. showed no inhibition of growth at 8 ppm concentration of pretilachlor and butachlor, however maximum growth was seen at the lower concentration. The organism showed 69%, 30%, 24% and 12% growth at 0.5 ppm, 1ppm, 4ppm and 8 ppm respectively on day 21. *Anabaena variabilis* showed growth even at 8 ppm concentration of pretilachlor and in presence of butachlor, it showed 126%, 33%, 33% growth in 0.5 ppm, 4ppm and 8 ppm respectively, on day 21 (Fig. 6). It was also reported in

Leptolyngbya sp. with 61%, 30% and 12% growth at 40, 60 and 80 ppm, respectively on day six (Singh *et al.*, 2014). The growth of *Synechocystis* sp. Strain PUPCCC 64 in graded concentrations (5–30 mg/L) of anilofos, concentration-dependent decrease in growth was observed (Singh *et al.*, 2013). Pretilachlor affected the growth of

Desmonostoc muscorum PUPCCC405.10 in a dose dependent manner with 15.8, 31.09, 65.0 and 76.09 % decrease in growth in 2.5, 5.0, 7.5, and 10 ppm of the herbicide in 6 days compared to control (Singh *et al.*, 2015).

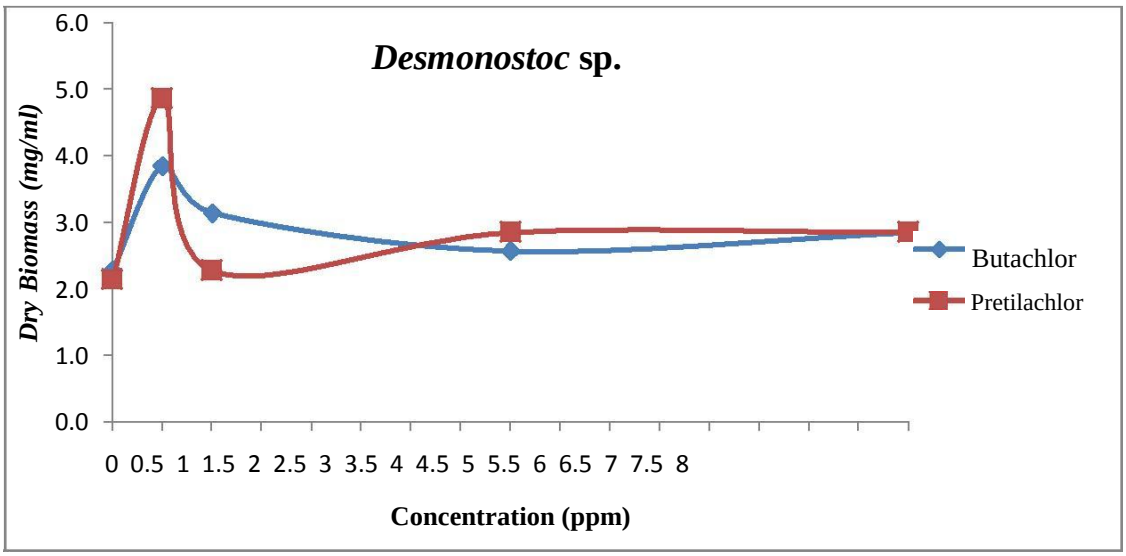

Fig. 5: Growth of *Desmonostoc* in the presence of pretilachlor and butachlor

There was decrease in the growth of *Desmonostoc* sp. up to 0.5 ppm, there after decline in the growth with increase in concentration of pretilachlor and butachlor (Fig. 5). Decline in growth was not much as compare to control i.e. *Desmonostoc* sp. can tolerate even high concentrations of herbicides. *Anabaena variabilis* showed stimulatory effect in the presence of pretilachlor at 0.5 ppm (Fig. 6) and in chase of butachlor stimulatory effect was observed at 1 ppm.

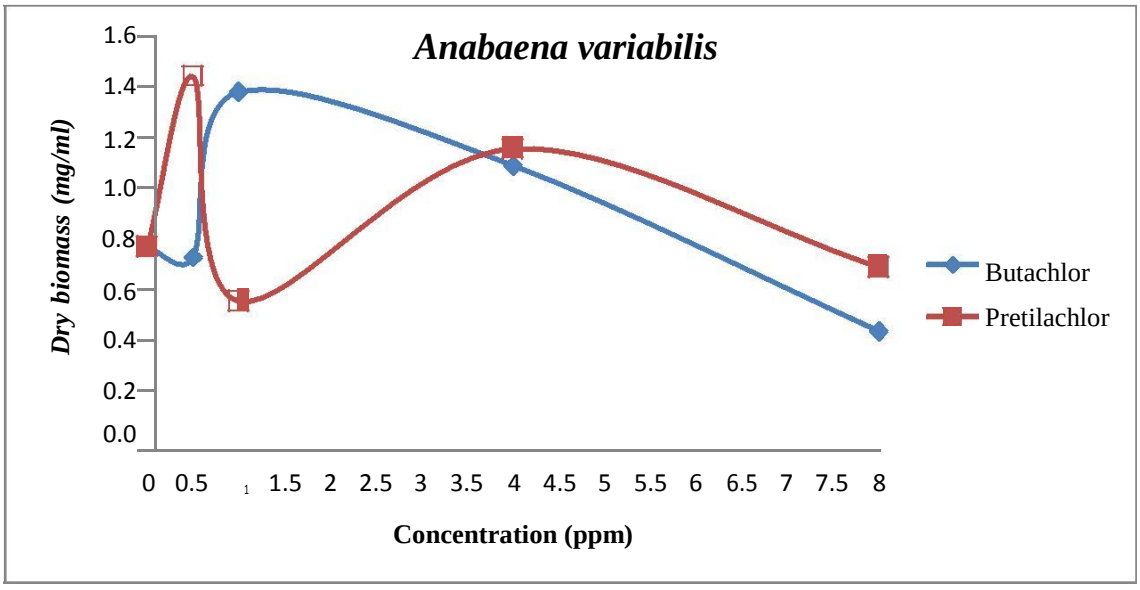

Fig 6: Growth of ARM-441 in the presence of butachlor and pretilachlor

The growth of *Anabaena* sp. ARM 441 in the presence of graded concentration of pretilachlor and butachlor, is shown in Fig. 6 where maximum growth was shown at lower concentration. ARM 441 showed maximum growth in presence of pretilachlor at 4 ppm, 43% increase in growth as compare to control and at 8 ppm showed decrease in growth by 49%. In presence of butachlor at 0.5 ppm showed 67.7 % increase in growth and at 8 ppm decreased by 38.9%.

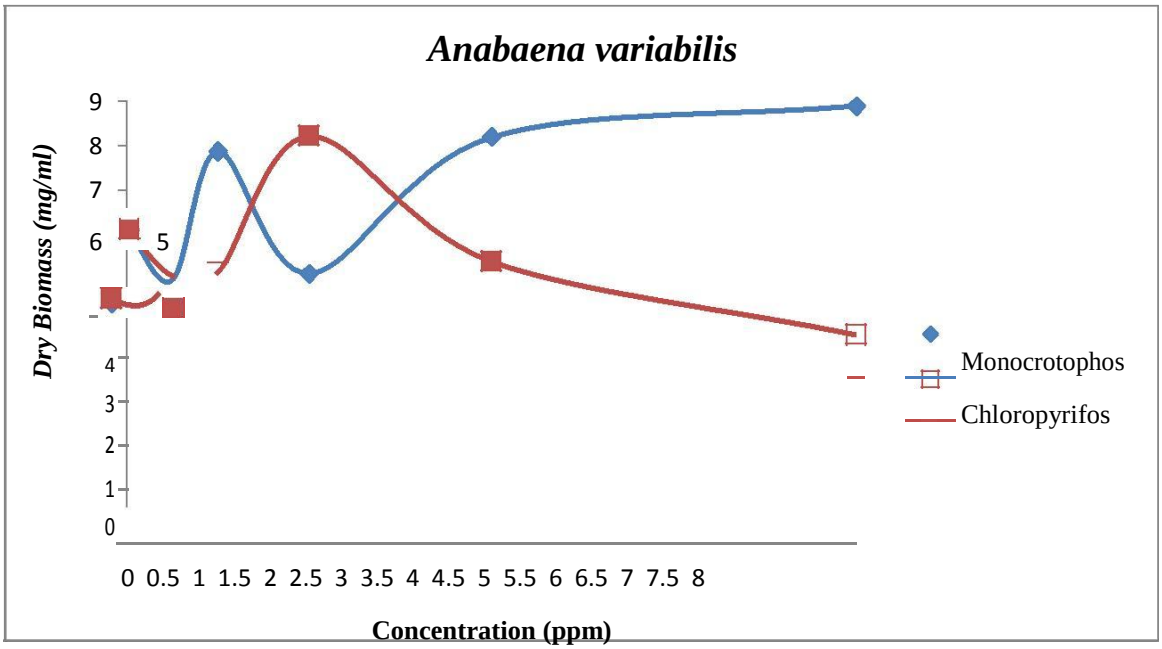

Fig. 7: The growth of *Anabaena viariabilis* in presence of graded concentration of monocrotophos and chlorpyrifos

Anabaena variabilis showed stimulatory growth at 1 ppm of monocrotophos and decrease in the growth at 2ppm was observed. However, stimulatory effect was also observed at high concentrations i.e. 4 ppm and 8 ppm (Fig. 7). In case of chlorpyrifos stimulatory growth was observed at 2 ppm (Fig. 7) and at higher concentrations there was decrease in the growth.

Desmonostoc sp. showed decline in growth at 0.5 ppm of chlorpyrifos and monocrotophos, however high concentrations (4 ppm and 8 ppm) showed increase in growth as compared to 0.5 ppm but was much less than the control.

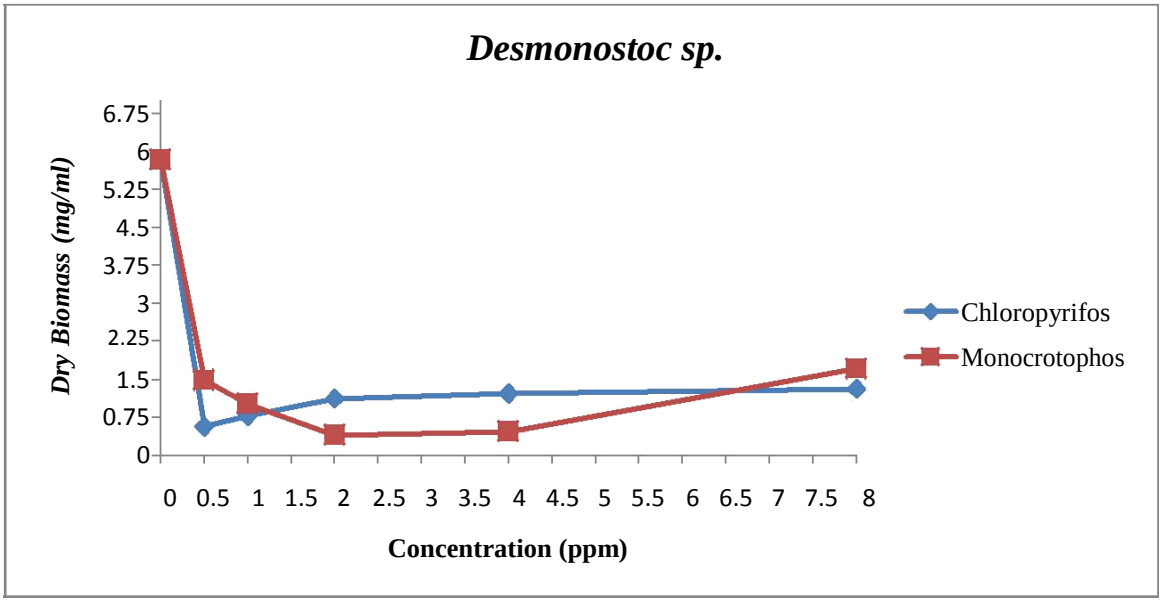

Fig. 8: Growth of *Desmonostocsp*. in presence of graded concentration of monocrotophos and chlorpyrifos

The growth of *Desmonostoc*in presence of graded concentration of monocrotophos and chlorpyrifos is shown in Fig. 8. At high concentration of chlorpyrifos and monocrotophos i.e. at 8 ppm maximum growth was observed. Lower concentration of chlorpyrifos inhibited the growth. At 8 ppm of monocrotophos, there was maximum growth but was less than the growth in control, overall there is inhibition of growth of *Desmonostoc*. There was decrease in growth by 90%, 86%, 80%, 79% and 77% at 0.5 ppm, 1 ppm, 2 ppm, 4 ppm and 8 ppm respectively. At 8 ppm of chlorpyrifos, *Desmonostoc* showed maximum growth but not more than control. The decrease in growth by 75%, 82%, 93%, and 70% was observed at 0.5 ppm, 1 ppm, 2 ppm, 4 ppm and 8 ppm pesticide respectively (Fig. 8).

4.2 Growth studies and Physiological studies

Growth studies were done in terms of biomass estimation and chlorophyll estimation.

Dry biomass estimation

Dry biomass estimation of cyanobacterial culture was done at different intervals of 7 days, 14 days and 21 days by Richmond and Gobbelaar (1986) method. Cyanobacterial

cultures were grown in different concentration of monocrotophos and chlorpyrifos (1, 4 and 8 ppm), pretilachlor and butachlor (1, 2 and 5 ppm) based on initial results.

Dry biomass estimation of *Anabaena variabilis*

In presence of herbicide there was inhibitory effect on the growth of *Anabaena variabilis*. In control there was maximum growth and as the concentration of pretilachlor increases the growth of cyanobacteria decreases. On day 7, the control had maximum growth and growth decreases by 13.7%, 30%, 45.6% at 1 ppm, 2 ppm, 5 ppm of pesticide respectively. On day 14th, percentage decrease in growth was 13.7%, 30%, 45.6% at 1 ppm, 2 ppm, 5 ppm at 1 ppm, 2ppm, 5 ppm respectively (Fig.9). Similar trend is followed up to 21 days and percentage decrease at day 21 was 14.7%, 29.4% and 42.1 % at 1, 2, and 5 ppm respectively. Similar result was reported by Fayez (2007), according to which the dry weight of *C. vulgaris* after seven days of 0.1 and 0.5 µM diuron treatments was lowered by 2.5 and 5.5 fold, respectively in comparison with the control.

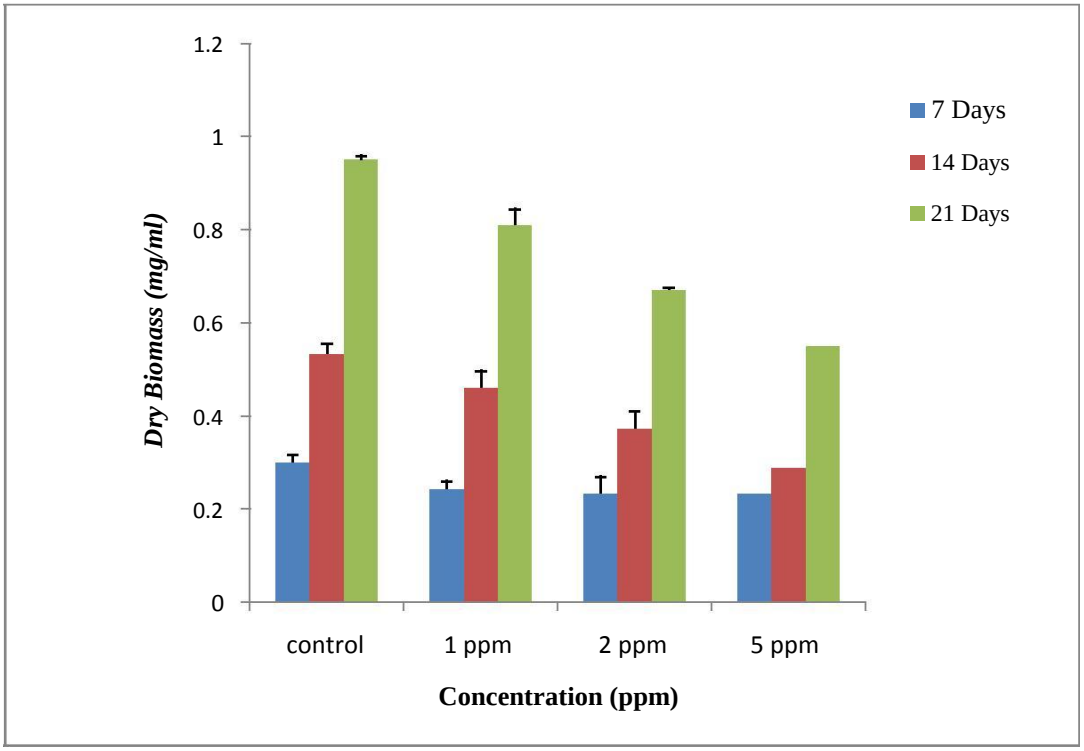

Fig. 9: Growth of *Anabaena variabilis* in presence of pretilachlor

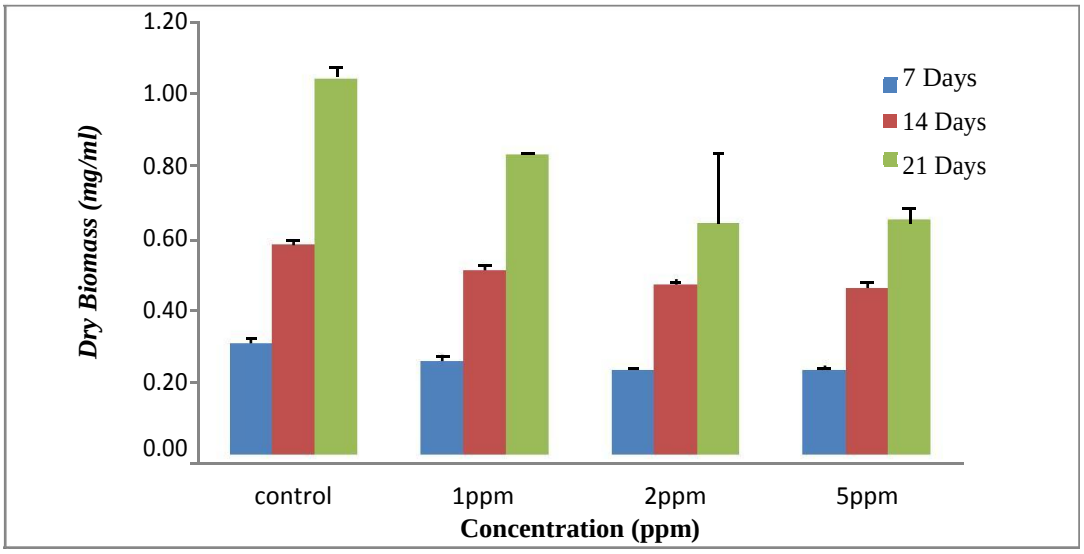

Fig. 10: Growth of *Anabaena variabilis* in presence of Butachlor

In presence of butachlor maximum growth was shown in case of control and presence of pesticide inhibits the growth of cyanobacteria. On 7th day percentage decrease in growth was observed to be 15%, 24% and 25% at 1 ppm, 2ppm, 5 ppm respectively. On day 14th, percentage decreases in growth was 12%, 19%, and 21% at 1 ppm, 2 ppm, 5 ppm of pesticide respectively. On day 21stpercentage decreases in growth was 20%, 38% and 37% at 1 ppm, 2 ppm, 5 ppm of pesticide respectively (Fig. 10).

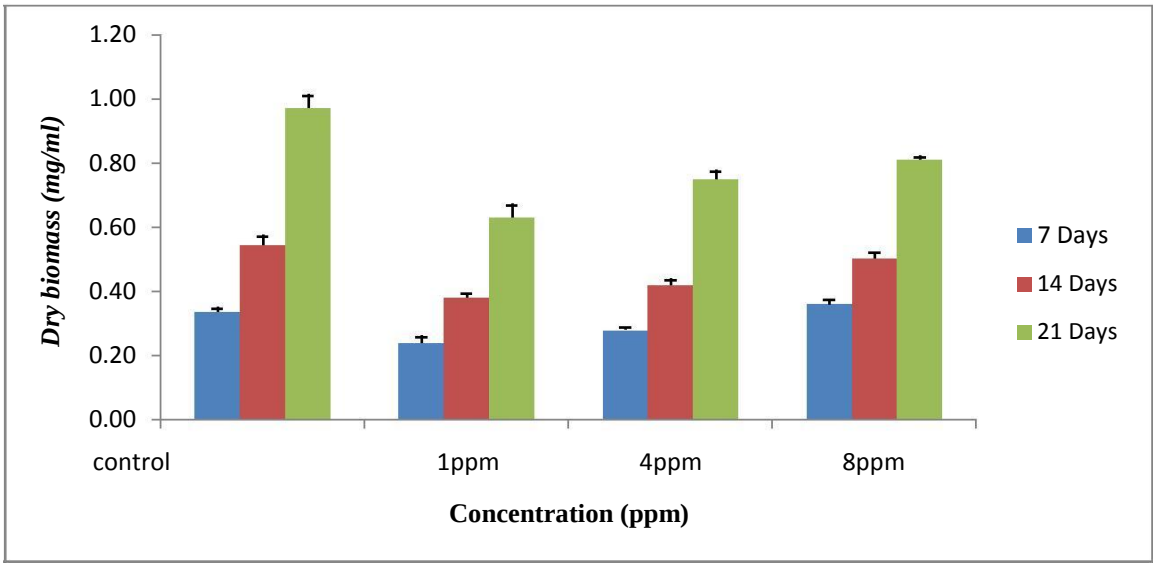

Fig. 11: Growth of *Anabaena variabilis* in presence of monocrotophos

In presence of monocrotophos maximum growth was shown by control. Except control maximum growth was observed in 8 ppm pesticide concentration and then followed by 4ppm and 1 ppm (Fig. 11). The percentage decrease in growth was 28% and 16% at 1 ppm and increase in 20% of growth at 8 ppm. On day 14th decrease in growth was 30%, 22% and 7% at 1 ppm, 4 ppm and 8 ppm respectively. On day 21st, there was decrease in biomass content and percentage decrease observed was 35, 22 and 16% at 1 ppm, 4 ppm and 8 ppm respectively.

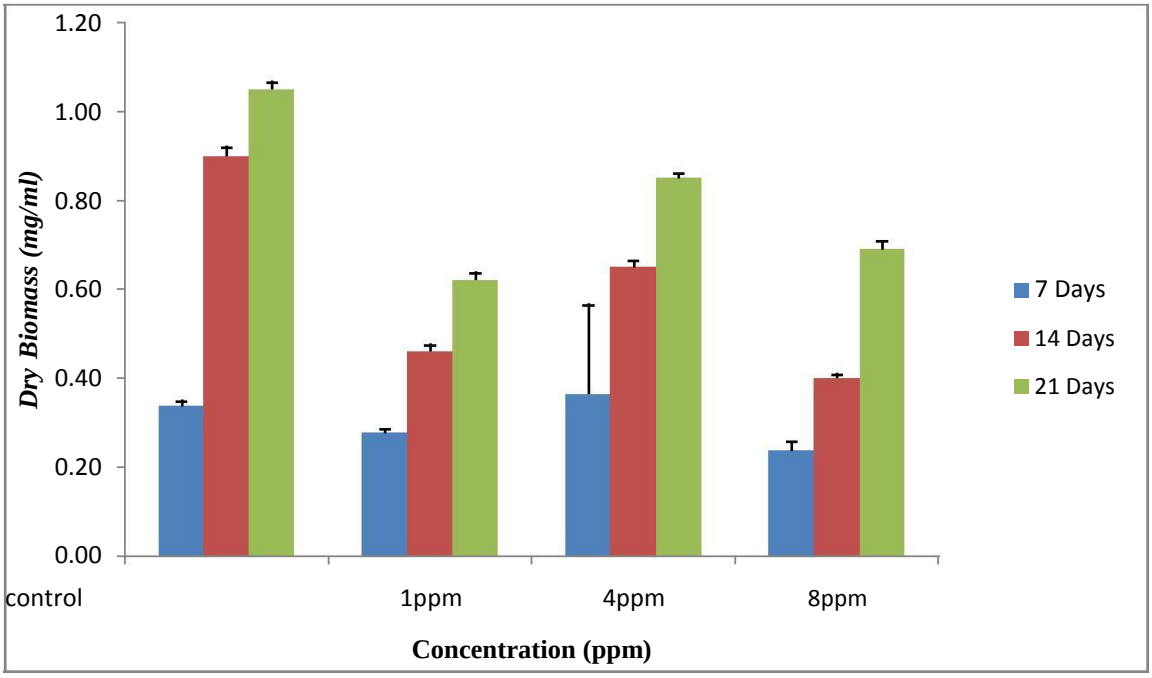

Fig. 12: Growth of *Anabaena variabilis* in presence of chlorpyrifos

In presence of chlorpyrifos, except control, culture withpesticide concentration 4 ppm showed maximum growth but not more than control (Fig. 12). After day 7, percentage decrease in growth was 40%, 19% and 34% at 1 ppm, 4 ppm and 8 ppm of pesticide respectively. At 14 day percentage decrease in growth was 48%, 27% and 55% at 1 ppm, 4 ppm and 8 ppm of pesticide respectively. At 21 day percentage decrease in growth was 40%, 19% and 34% at 1 ppm, 4 ppm and 8 ppm of pesticide respectively (Fig. 12). Similar results were reported by Wong in 2000, which proved that the presence of 0.02, 0.2

or 2 mg/l of 2, 4-D was not toxic to the alga. Algal growth, photosynthesis and chlorophyll-a synthesis were stimulated by the presence of concentrations (0.02 or 0.2 and 0.02 mg/l, respectively) of 2, 4-D and glyphosate.

Dry biomass estimation of *Desmonostoc*

Desmonostoc sp. in presence of pretilachlor there was no inhibitory effect at 1 ppm but at higher concentration growth was declined (Fig.13). In case of butachlor there was stimulatory effect on the growth and at higher concentration there was decline in growth (Fig.14). Monocrotophos and chlorpyrifos showed stimulatory effect on the growth on 1 ppm and inhibitory effect on high concentration.

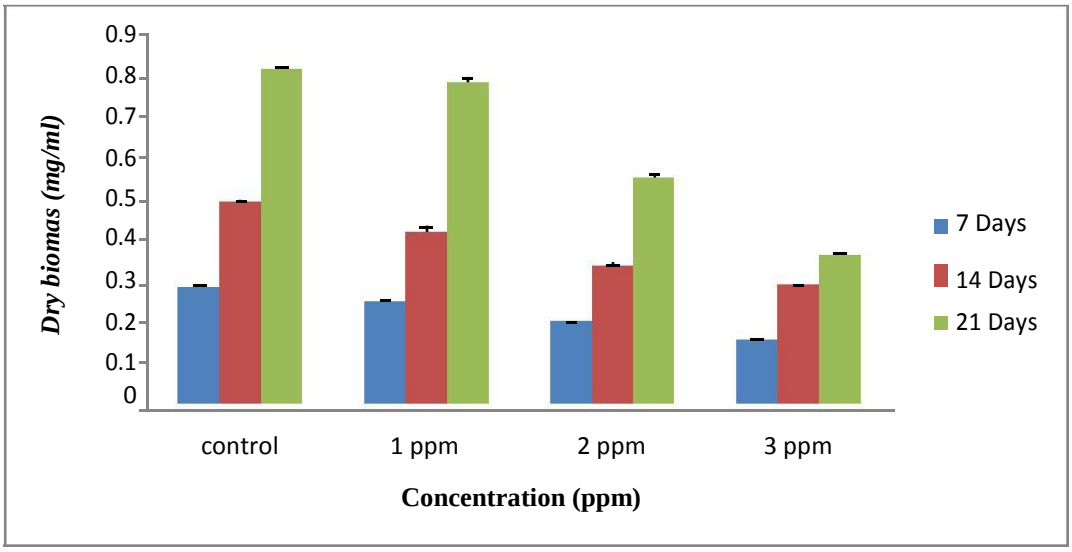

Fig. 13: Growth of *Desomonostoc sp*. in presence of pretilachlor

In presence of pretilachlor there was decrease in growth of cyanobacteria as the concentration of pesticide increases. On 7th day percentage decrease in biomass was 12%, 33% and 63% in comparison to control at 1ppm, 2 ppm and 5ppm of pretilachlor. On 14th day percentage decrease in biomass was 14%, 37% and 59% as compared to control at 1ppm, 2 ppm and 5ppm respectively. In presence of butachlor maximum growth was observed at 1 ppm at day 21(Fig.13).

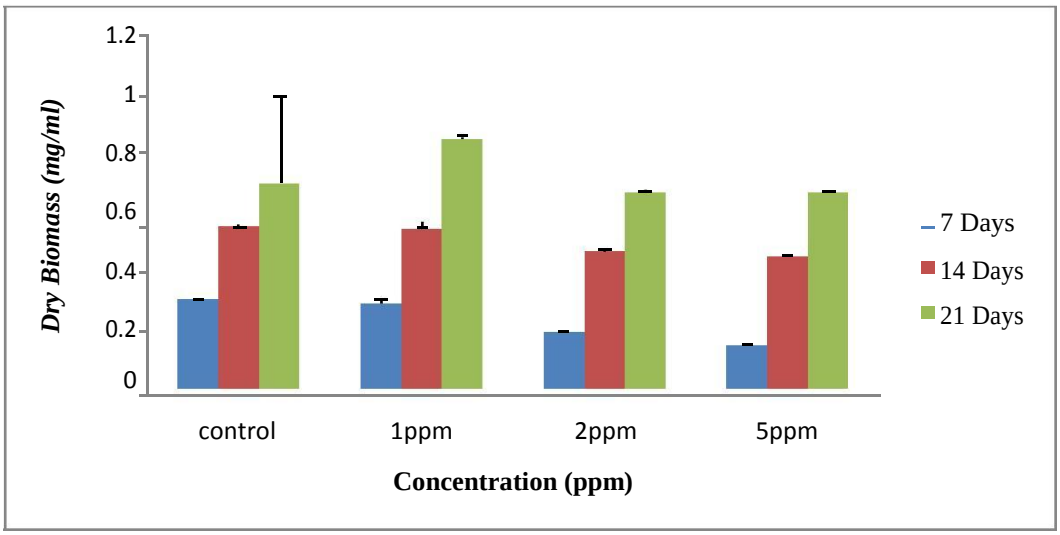

Fig. 14: Growth of *Desmonostocsp.* in presence of butachlor

On day 7 percentage decreases in growth was 4%, 37% and 51% as compare to control at 1ppm, 2 ppm and 5ppm of butachlor respectively. On 14 day percentage decrease in growth was 1%, 15% and 18% as compare to control at 1ppm, 2 ppm and 5ppm of butachlor respectively. On 21st day at 1 ppm growth was stimulated by 22% and declined at growth at 2 and 5ppm (Fig. 14).

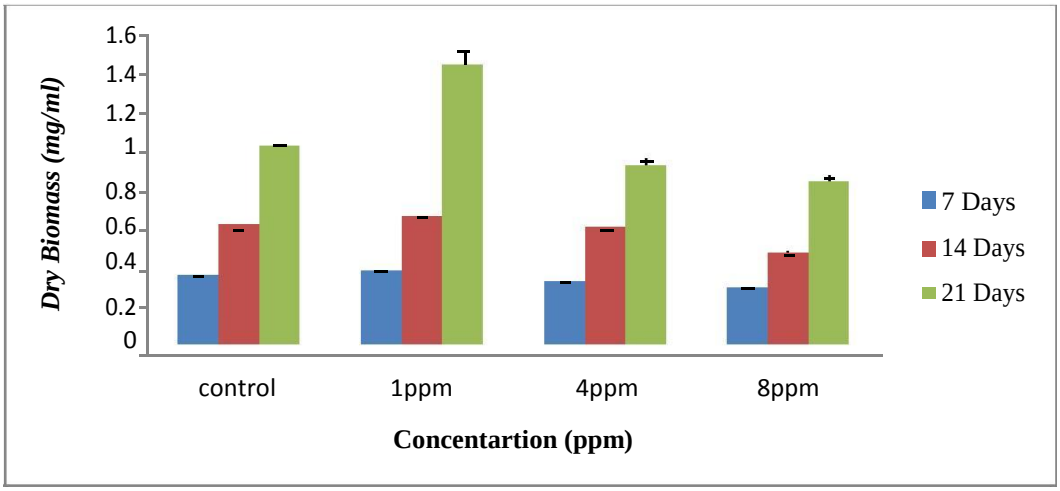

Fig. 15: Growth of *Desmonostocsp.* in presence of monocrotophos

At 1 ppm increase in biomass was 6.5 % after 7 and 14 days and 40% after 21 days. At 4 ppm and 8 ppm biomass decreased. Percentage decrease was 9%, 2% and 10% at 4 ppm after interval of 7 days each and 18%, 24% and 17% after 7, 14 and 21 days respectively at 8 ppm (Fig. 15) .

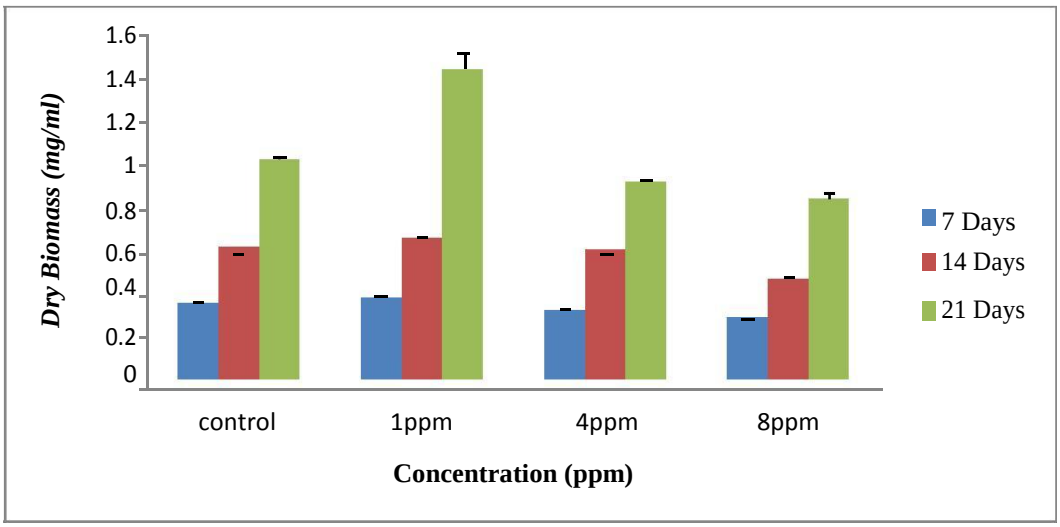

Fig. 16: Growth of *Desmonostocsp.* in presence of chlorpyrifos

In presence of chlorpyrifos, at 1 ppm and 4 ppm there was stimulation as compared to control. Percentage increase in biomass was 41% and 20% at 1 ppm and 4 ppm after 7 days and decrease in biomass was 12% at 8 ppm. Percentage increase in biomass was 30% and 6% at 1 ppm and 4 ppm after 14 days and decrease in biomass 29% at 8 ppm. After 21 days, it was observed that percentage increase in biomass was 11% and 2% at 1 ppm and 4 ppm and decrease in biomass 12% at 8 ppm (Fig. 16). Overall results showed that lower concentration of herbicides and insecticides enhance the growth of cyanobacteria.

Estimation of chlorophyll

Anabaena variabilis in presence of pretilachlor and butachlor showed decline in chlorophyll content as the concentration of pesticides increase (Fig. 17, 18), but there was not much decline in the chlorophyll content i.e. *Anabaena variabilis* can tolerate high concentration of pesticides. In case of monocrotophos at 8 ppm stimulatory effect on the growth, maximum chlorophyll content was observed (Fig. 19). Chlorpyrifos showed decline in growth at 1 ppm, however increase in growth was observed at 4 ppm (Fig. 20).

Estimation of chlorophyll in *Anabaena variabilis*

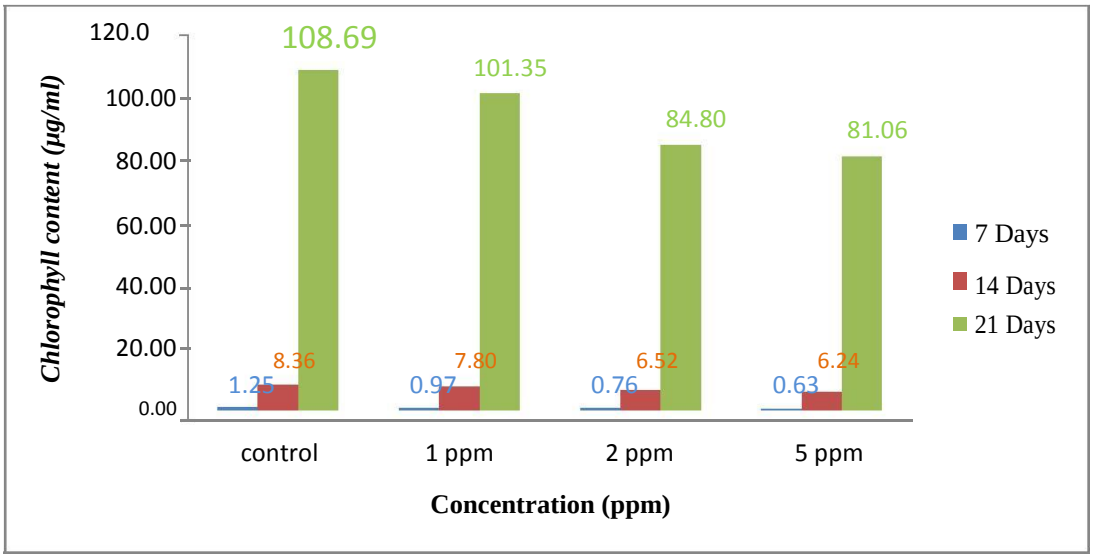

Fig. 17: Chlorophyll estimation in presence of pretilachlor

In presence of pretilachlor as the concentration of pesticide increases the chlorophyll content of cyanobacteria decreases by 21%, 29% and 39% on 7th day at 1 ppm, 2ppm and 5 ppm respectively. On 14th and 21st day percentage decrease was 6%, 21% and 25% at 1 ppm, 2ppm and 5 ppm respectively (Fig. 17). Same kind of result was observed by Fayez in 2007 in case of *C. vulgaris* in presence of diuron where at lowest concentration (0.1 µM) chlorophyll content was 3.32 µg/ml and at higher concentration (0.5 µM) was 0.98 µg/ml as control was 7.45 µg/ml.

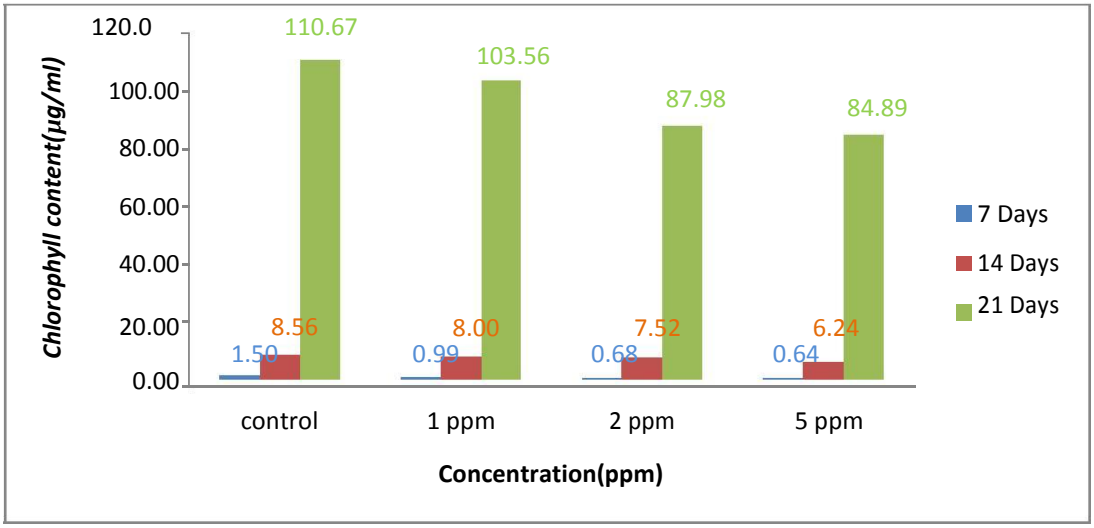

Fig. 18: Chlorophyll estimation in presence of butachlor

In case of butachlor, as the concentration of pesticide increases the chlorophyll content of cyanobacteria decreases by 34%, 54% and 57% on 7th day at 1 ppm, 2ppm and 5 ppm respectively. After 14thday, percentage decrease was 6%, 12% and 27% at 1 ppm, 2ppm and 5 ppm respectively. After day 21st percentage decrease was 6%, 20% and 23% at 1 ppm, 2ppm and 5 ppm respectively (Fig. 18).

Mild stimulation of photosynthesis was observed by Chen *et al* in 2007 over a limited range of low concentrations of these three pesticides. We found that PSII and PSI were, respectively, the inhibitory sites of 150 µM butachlor and 150µM bensulfuron-methyl.

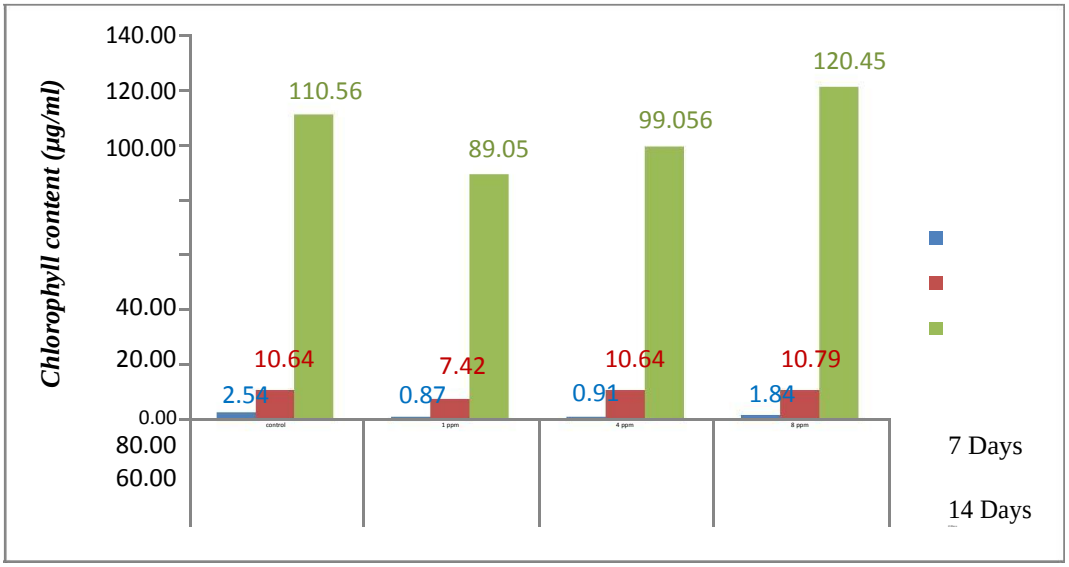

Fig. 19: Chlorophyll estimation in presence of monocrotophos

On 7th day percentage decrease in chlorophyll was 65%, 64% and 27% at 1 ppm, 4 ppm and 8 ppm of monocrotophos respectively. On day 14th it was observed that 8 ppm stimulated the growth by 1.5% and at 1 ppm and 4 ppm chlorophyll content decreased by 30% and 0.02%. After 21 days percentage decrease was 19% and 10% at 1 ppm and 4 ppm and increase in chlorophyll content at 8 ppm by 10% (Fig. 19).

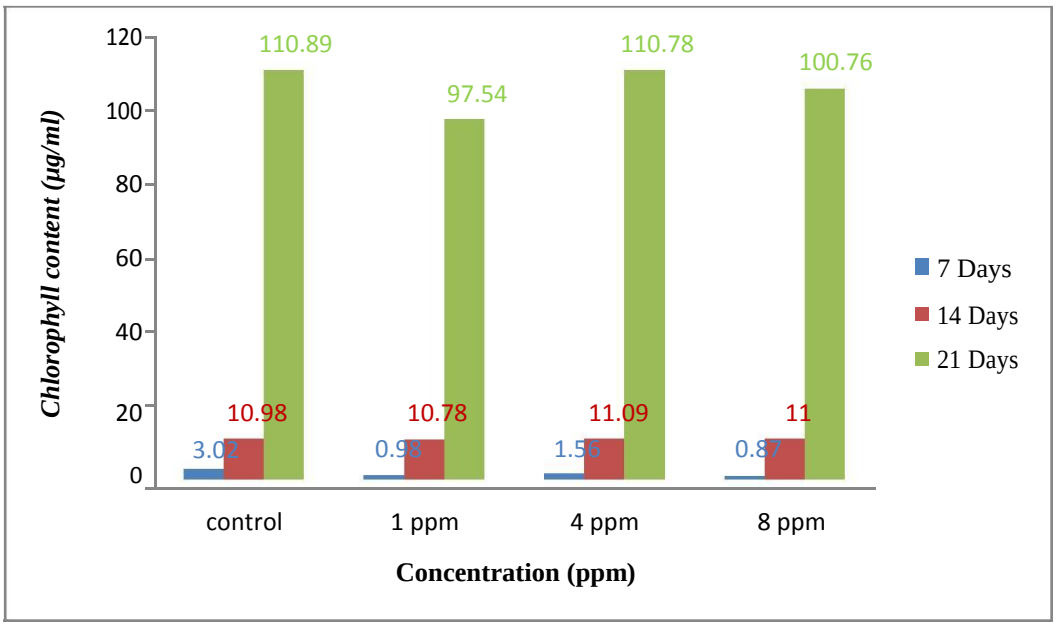

Fig. 20: Chlorophyll estimation in presence of Chlorpyrifos

In case of chlorpyrifos, higher concentration stimulated the growth of cyanobacteria. After 7 days overall chlorophyll content was decreased by 67%, 48% and 71% at 1 ppm, 4 ppm and 8 p pm respectively. After 14 days there was increase in chlorophyll content by 1% and 0.015 at 4 ppm and 8 ppm and at 1 ppm decrease by 1.8%. On 21st day maximum growth was shown at 8 ppm i.e. 110.78 ug/ml (Fig.20). The presence of anilofos caused an inhibitory effect on the growth of *Synechocystis* sp. strain PUPCCC 64 in a concentration dependent manner. After 6 days there was decrease in 41% and 61% over control with 10 and 20 mg/ml of anilofos (Singh *et al.*, 2013).

Estimation of chlorophyll in *Desmonostoc sp.*

*Desmonostoc*sp. in presence of pretilachlor had inhibitory effect on the growth, as the concentration of pretilachlor increases, there was decline in the chlorophyll content (Fig. 21). Similar kind of trend was followed in presence of butachlor (Fig. 22). In case of monocrotophos there was stimulatory effect on the growth at 1 ppm (Fig. 23). Chlorpyrifos showed stimulatory effect at 1 ppm and at 8 ppm there was no inhibition of growth (Fig. 24).

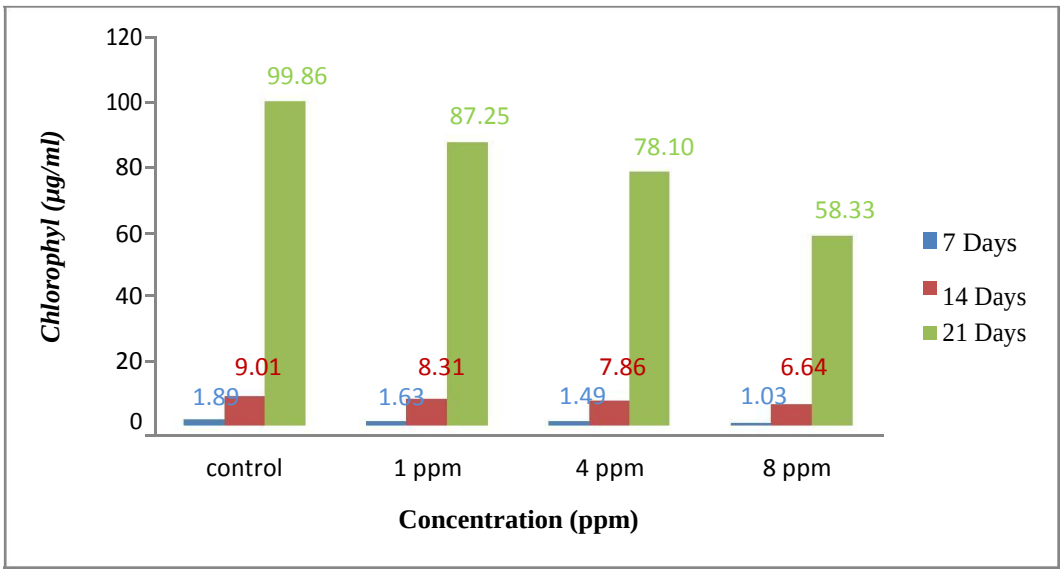

Fig. 21: Chlorophyll estimation in presence of pretilachlor

In presence of different concentration of pretilachlor, growth of cyanobacteria was inhibited effect at higher concentration, as the concentration of pesticide increased chlorophyll content was decreased. After 7 days percentage decline in chlorophyll content was 13%, 21% and 45% at 1ppm, 2 ppm and 5 ppm of pesticide respectively. After 14 days decrease in chlorophyll content was 7%, 12% and 26% at 1ppm, 2 ppm and 5 ppm of pesticide respectively. After 21 days percentage decrease in chlorophyll content was 12%, 24% and 53% at 1ppm, 2 ppm and 5 ppm of pesticide respectively (Fig. 21).

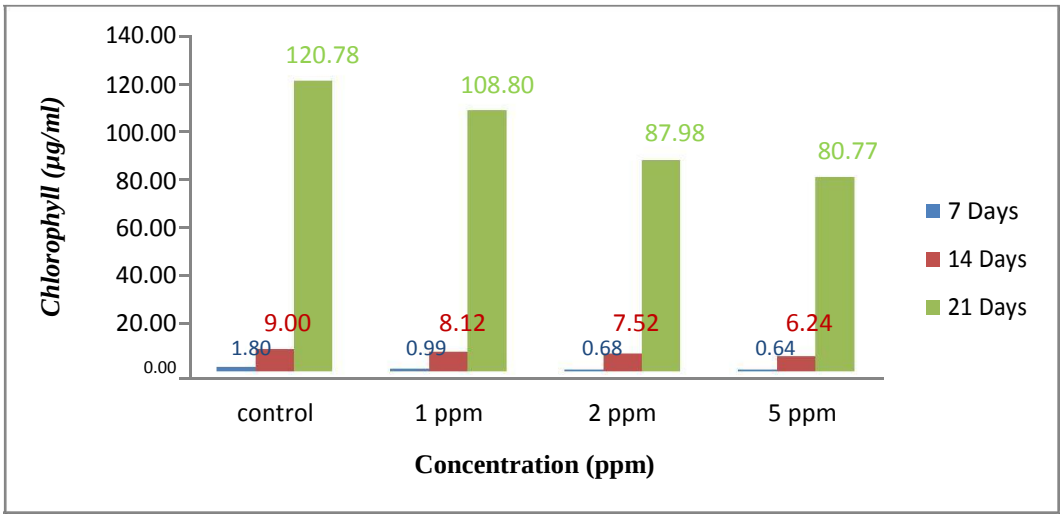

Fig. 22: Chlorophyll estimation in presence of butachlor

In presence of butachlor, chlorophyll content decreases as concentration of pesticide increases. After 7 days percentage decrease in chlorophyll content was 45%, 113% and 117% at 1ppm, 2ppm and 5ppm of pesticide respectively. After 14 days decrease in chlorophyll content was 9%, 16% and 30% at 1ppm, 2ppm and 5ppm of pesticide respectively. After 21 days percentage decrease in chlorophyll content was 9%, 27% and 33% at 1ppm, 2ppm and 5ppm of pesticide respectively (Fig. 22) Same kind of trend was observed by Tariya and Dubey in 2014, at higher concentration (300 ppm) concentration of Nomnigold (herbicide, bispyribac sodium) decrease the chlorophyll content of

Anabaena circinalis, Scytonemahofmanni, Aulosira prolific and *Tolypothrix fragilis* by 68%, 35.5 %, 13.4% and 14.6% respectively.

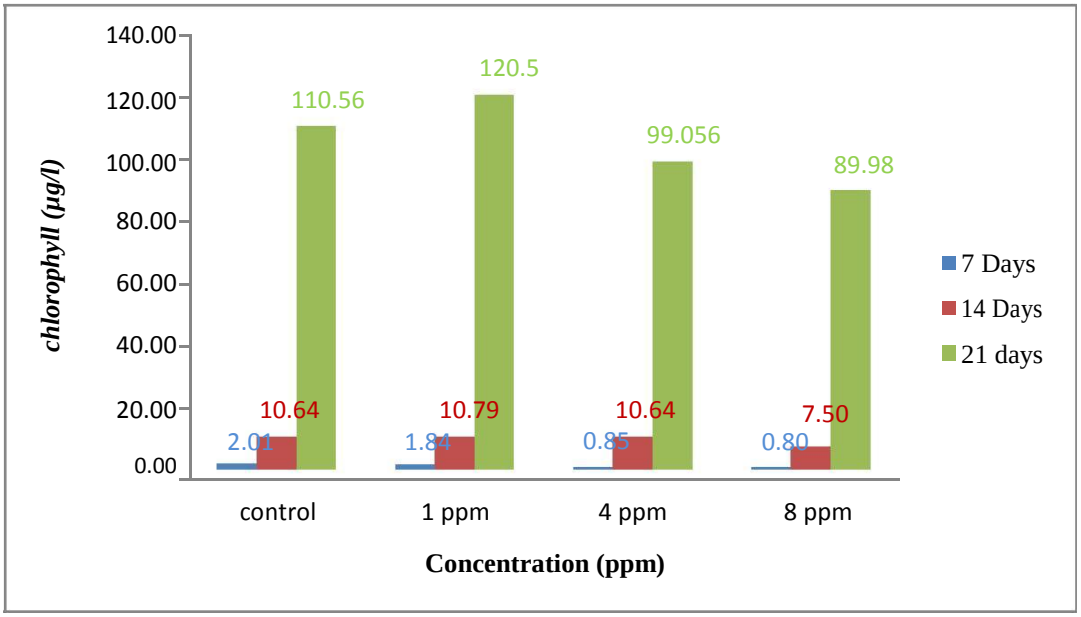

Fig. 23: Chlorophyll estimation in presence of monocrotophos

In presence of monocrotophos, it was observed that lower concentration of pesticide increased the chlorophyll content as compared to control. Increase in chlorophyll content was 2% and 9% after 14 and 21 days respectively at 1 ppm concentration, decrease in chlorophyll content after 7 days was 8%, 57%, 60% at 1ppm, 4 ppm and 8 ppm respectively and after 14 days 0.02% and 29% at 4 ppm and 8 ppm respectively. After 21 days decrease in chlorophyll content was 10 and 18% at 4 ppm and 8 ppm respectively (Fig. 23).

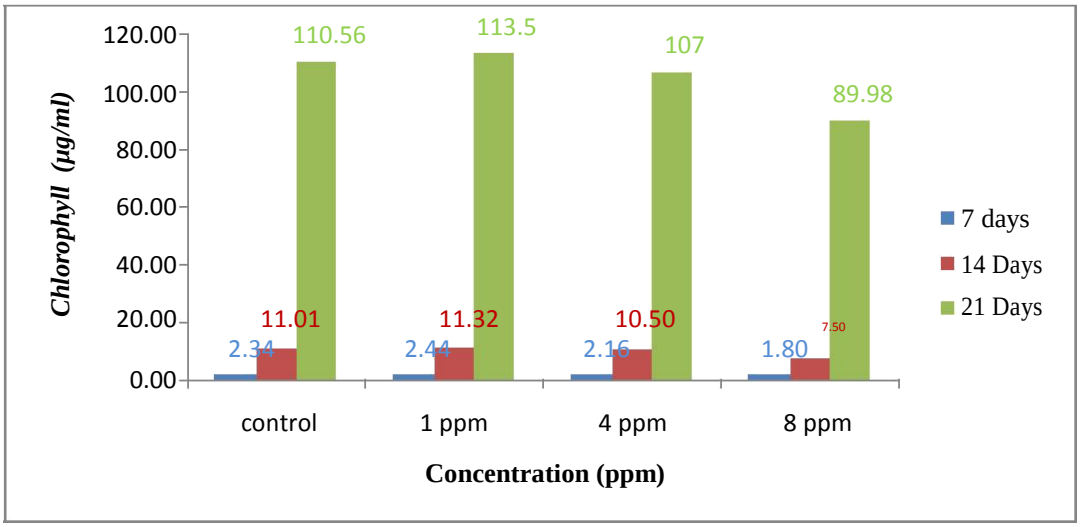

Fig. 24: Chlorophyll estimation in presence of chlorpyrifos

In presence of chlorpyrifos chlorophyll content was increased at lower concentration and decreased at higher concentration At 1 ppm increase in chlorophyll content was 4.2%, 2.6% and 2.6% after 7, 14 and 21 days respectively. At 4 ppm decrease in chlorophyll was 7%, 3% and 3% over control after 7, 14 and 21 days.At 8 ppm decrease in chlorophyll was 23%, 18% and 19% over control after 7, 14 and 21 days (Fig. 24). Increase in chlorophyll content of *Nostoc sp*. was observed by Chen *at el* in 2007 in presence of low concentrations of butachlor, bensulfuron-methyl. Percentage increase in comparison to control was 28% and 7% at 10 and 100 µM of butachlor and 2.5% increase at 100 µM of bensulfuron-methyl.

4.3 Carbohydrate Estimation

Total carbohydrate estimation was done of cyanobacterial cultures grown in different concentration of pesticides and herbicides.

Carbohydrate Estimation of *Anabaena variabilis*

On day 7 in presence of pretilachlor, butachlor, monocrotophos and chlorpyrifos there was no significant amount of carbohydrates produced.

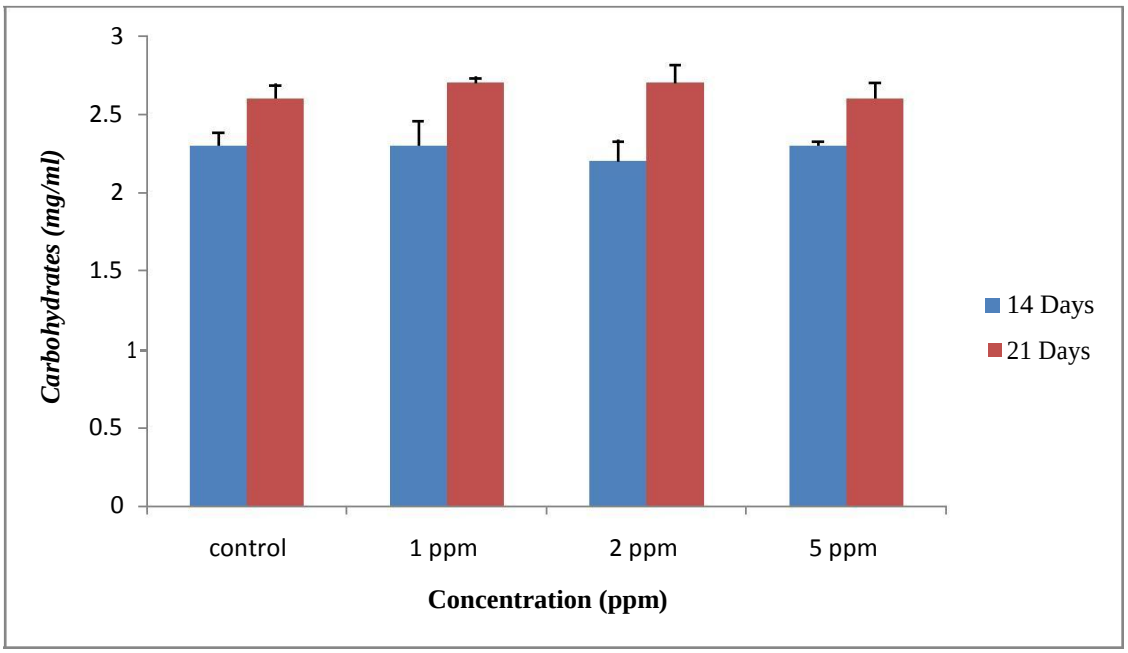

Fig. 25: Carbohydrate content in presence of pretilachlor

On day 14 amount carbohydrate produced was 2.5 mg/ml which is slightly greater than the control (Fig. 25). Overall there was no effect on the carbohydrate production in presence of herbicides.

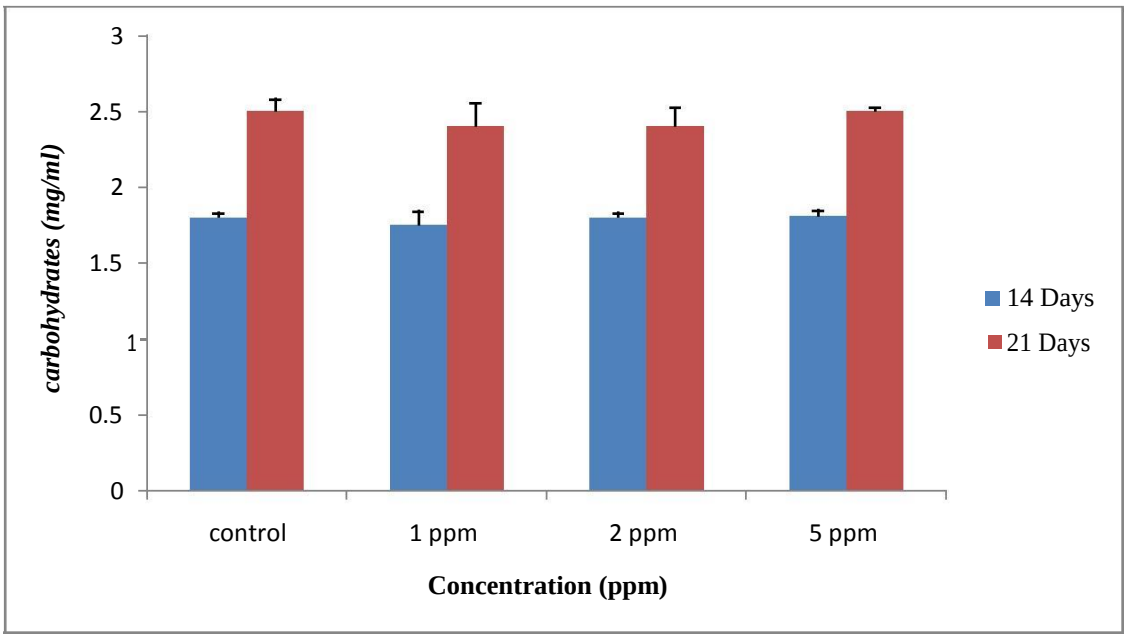

Fig. 26: Carbohydrate content in presence of butachlor

Carbohydrate content remained constant in each concentration i.e. 1, 2, 5 ppm but there was slight increase in the content of carbohydrates depicted that in presence of herbicides produce large amount of carbohydrates as compare to control (Fig. 25, 26). Similar kind of results was observed in case of monocrotophos (Fig. 27) and chlorpyrifos (Fig. 28).

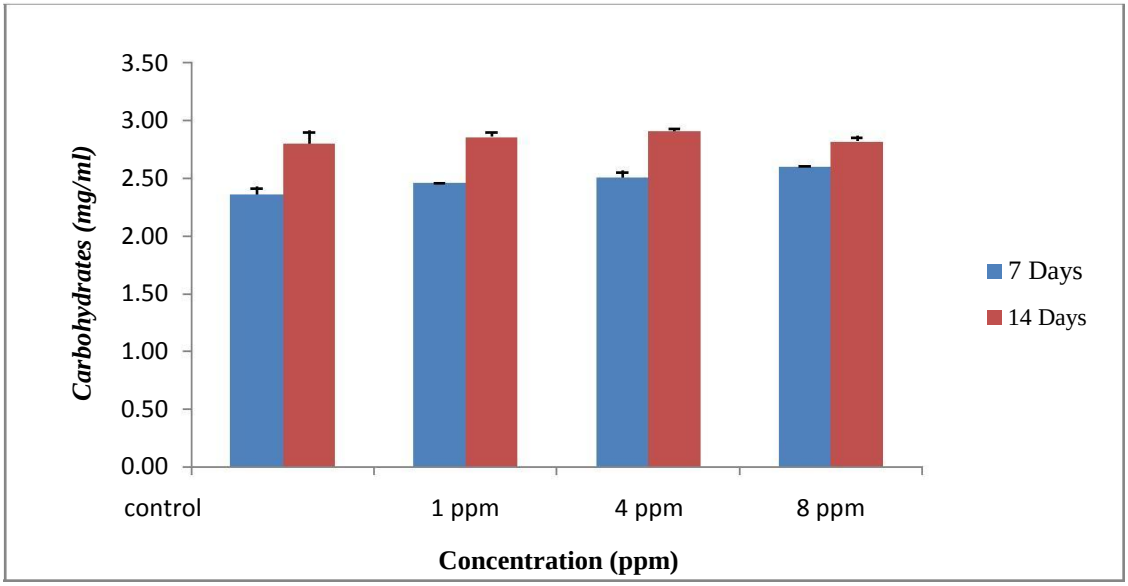

Fig. 27: Carbohydrate content in presence of monocrotophos

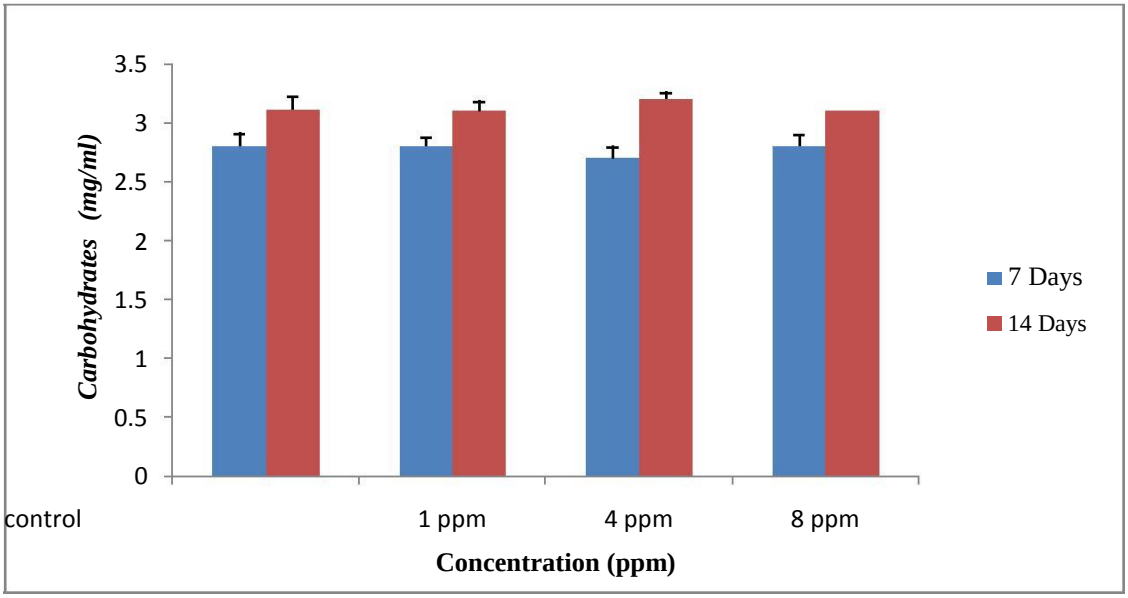

Fig. 28: Carbohydrate content of chlorpyrifos

In case of *Anabaena variabilis* ARM 441, there was no carbohydrate produced at 7 days. On day 14 and 21 there was no effect on the carbohydrate content in presence of pretilachlor, butachlor, nonocrotophos and chlorpyrifos there was no change in content of chlorophyll but that was slightly more than control, i.e. in presence of insecticides and herbicides *Anabaena variabilis* produce more amount of carbohydrates as compare to control.

Carbohydrate Estimation in *Desmonostoc sp.*

Desmonostoc sp. in presence of high concentration of herbicides (2 and 5 ppm) and insecticides (4 and 8 ppm) showed decrease in the carbohydrate content, whereas at low concentration i.e. 1 ppm increase in carbohydrate content was observed (Fig. 29, 30, 31, 32).

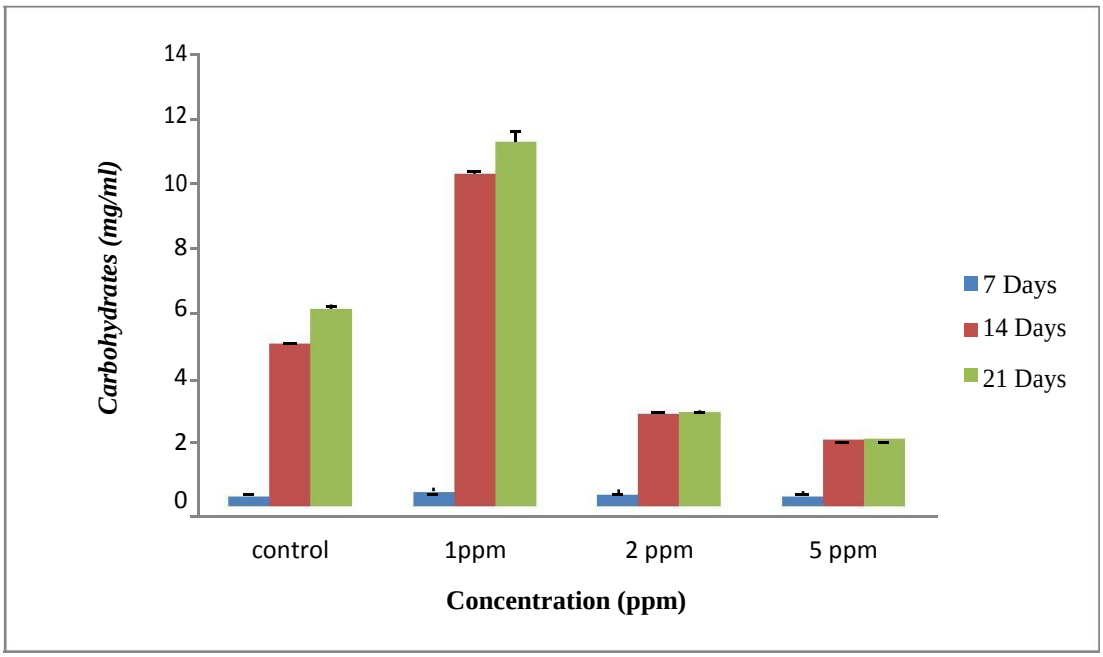

Fig. 29: Carbohydrate content in presence of pretilachlor

In presence of pretilachlor maximum carbohydrate content was shown at 1 ppm i.e. 0.5, 10.29 and 11.29 mg/ml after day 7, 14 and 21 respectively. At 2 and 5 ppm carbohydrate content was decreased by 42% and 59% after 14 days. After 21 days carbohydrate content was decreased by 52% and 65% at 2 ppm and 5 ppm respectively (Fig. 29).

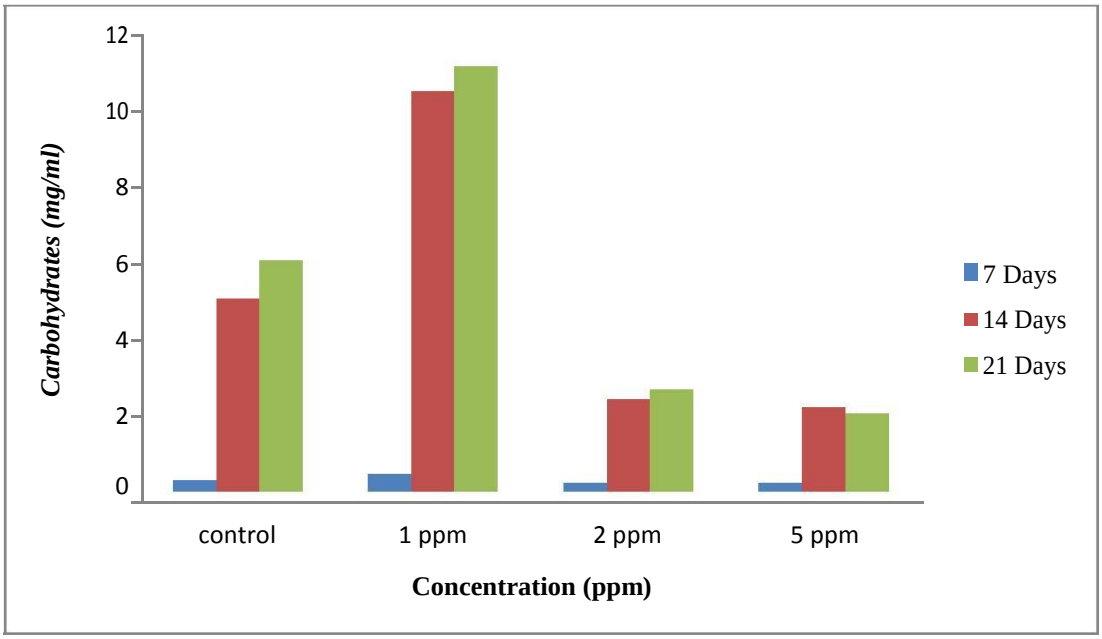

Fig. 30: Carbohydrate content in presence of butachlor

In case of butachlor same kind of result was noted, carbohydrate content was maximum at 1 ppm i.e. 0.47, 10.5, 11.1 mg/ml after 7, 14 and 21days. At 2 ppm and 5 ppm decrease in carbohydrate content was 51% and 55% after 14 days. After 21 days 55% and 65% decrease in carbohydrate content at 2 ppm and 5 ppm (Fig. 30).

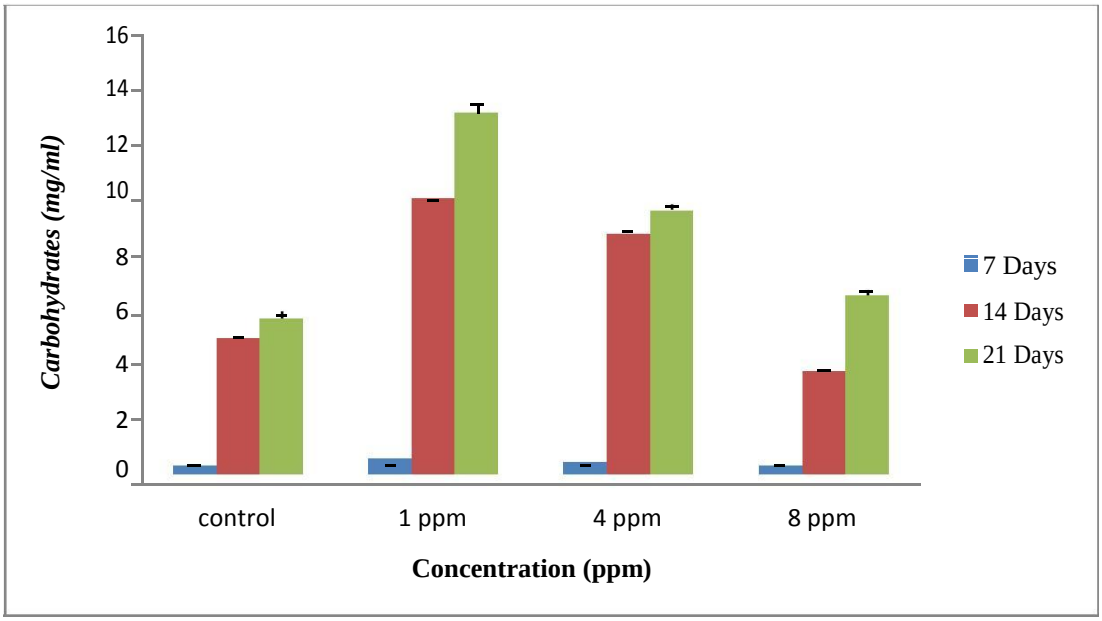

Fig. 31: Carbohydrate content in presence monocrotophos

Carbohydrate content was maximum at 1 ppm i.e. 0.47, 10.5, 11.1 mg/ml after 7, 14 and 21days. In presence of monocrotophos algae showed increase in the carbohydrate content as compared to the control percentage increase after 7 days was 78% and 36% at 1 ppm and 4 ppm respectively. After 14 days increase was 104%, 77.5% increase at 1 ppm and 4 ppm respectively and at 21 days percentage increase was 146%, 69% and 16% at 1 ppm, 4 ppm and 8 ppm respectively (Fig. 31).

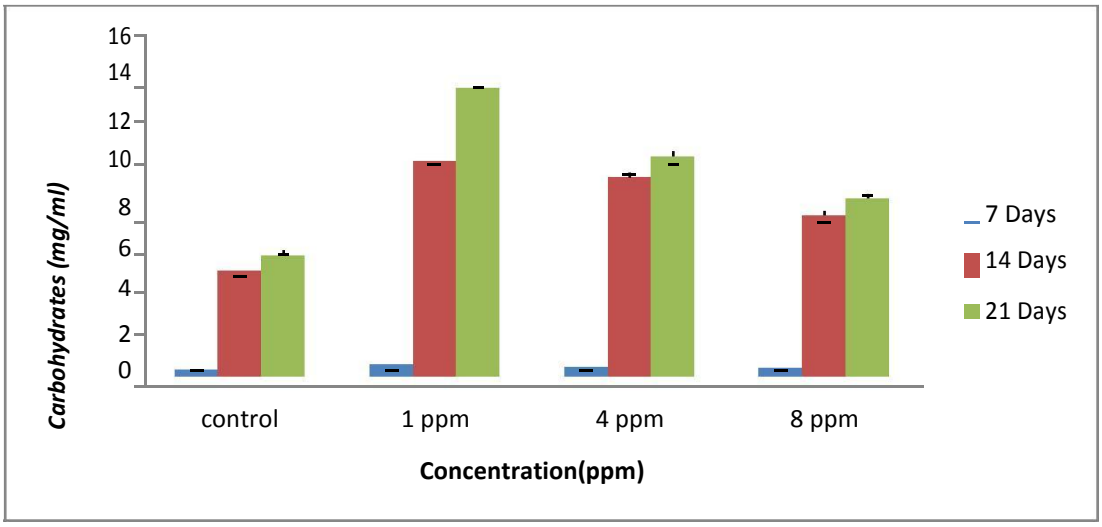

Fig. 32: Carbohydrate content in presence of chlorpyrifos

In presence of Chlorpyrifos percentage increase in carbohydrate content were 78%, 36% and 31% increase after 7 days at 1 ppm, 4ppm and 8 ppm. After 14 days increase in carbohydrate content was 104%, 88% and 53% at 1 ppm, 4ppm and 8 ppm respectively. 138%, 81% and 47% increase in carbohydrate content after 21 days at1 ppm, 4ppm and 8 ppm respectively (Fig. 32). Carbohydrates were enhanced in cells grown in pesticide-amended medium. Same kind of results was reported in case of *T. scytonemoides*by Rajendran in 2007 when grown in 0.2 ppm monocrotophos exogenous maximum amount of carbohydrates were released and when treated with 0.5 ppm of nimbicidin enhance the release of carbohydrates.

4.4 Nitrate Reductase Activity

Nitrate reductase activity decreases in case of *Anabaena variabilis* as the concentration of herbicide and insecticide increases.

Nitrate reductase activity in *Anabaena variabilis*

In case of *Anabaena variabilis*, similar kind of effect of herbicides and insecticides on the nitrate reductase activity was observed.

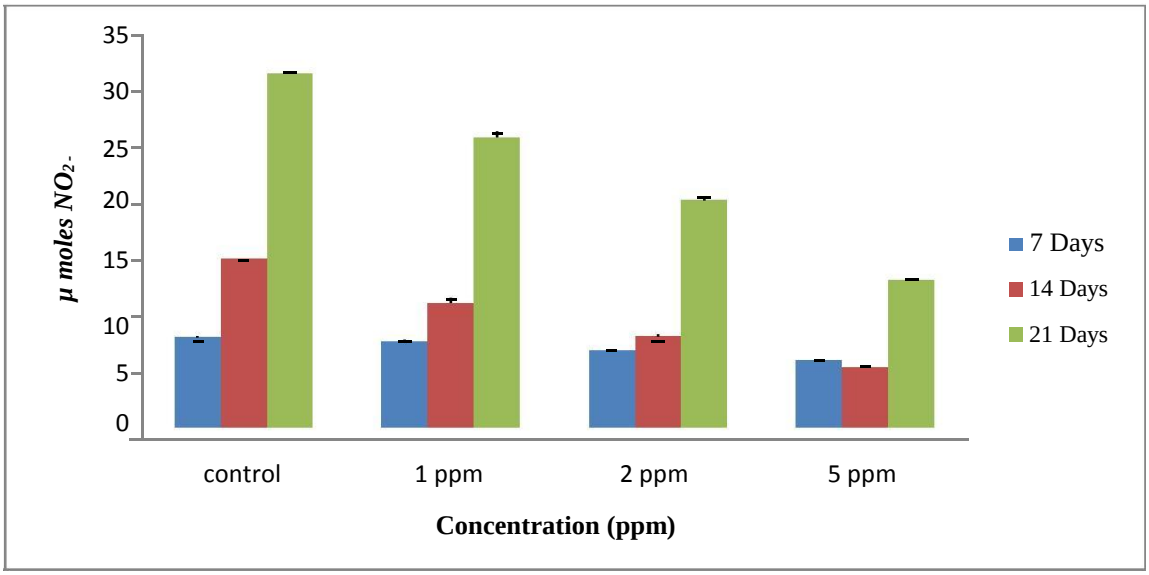

Fig. 33: Nitrate reductase activity in presence of pretilachlor

As the concentration of pretilachlor increases nitrate reductase activity decreases. At 1 ppm nitrate reductase activity was 7.6, 11.09 and 25.8 µmoles after 7, 14 and 21 days respectively. At 5 ppm nitrate reductase activity was 6.02, 5.39 and 13.14 µmoles after 14 and 21 days respectively (Fig. 33).

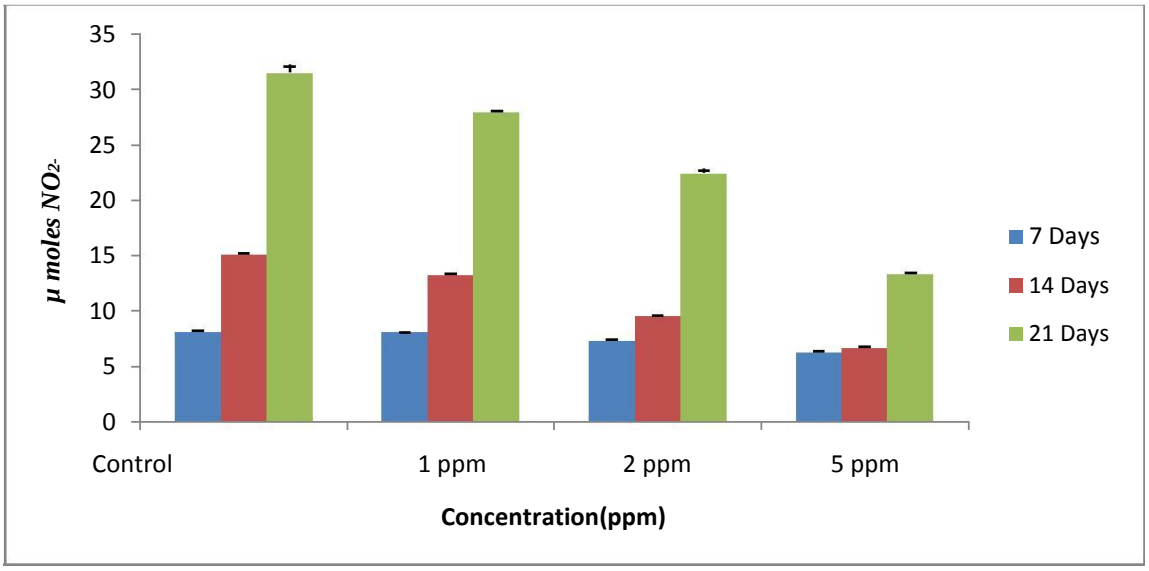

Fig. 34: Nitrate reductase activity in presence of butachlor

In presence of butachlor, at 1ppm nitrate reductase activity was 8.04, 13.25 and 27.87 µmoles after 7, 14 and 21 days respectively. At 5 ppm nitrate reductase activity was 6.25, 6.65 and 13.29 µmoles after 14 and 21 days respectively (Fig. 34).

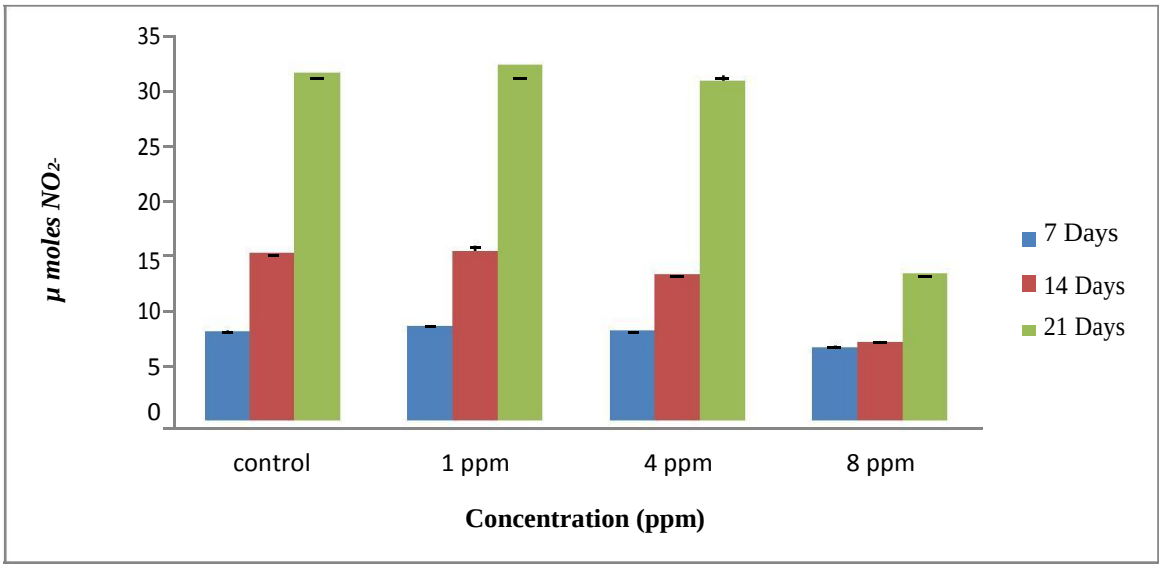

Fig. 35: Nitrate reductase activity in presence of monocrotophos

In presence of monocrotophos 1 ppm enhanced the activity of nitrate reductase by 5.57%, 1.02% and 2.38% respectively 7, 14 and 21 days as compared to control. At 4 ppm decrease in nitrate reductase activity was 12% and 53% after 14 and 21 days. At 8 ppm percentage decrease was 2% and 58% after 14 and 21 days (Fig. 35).

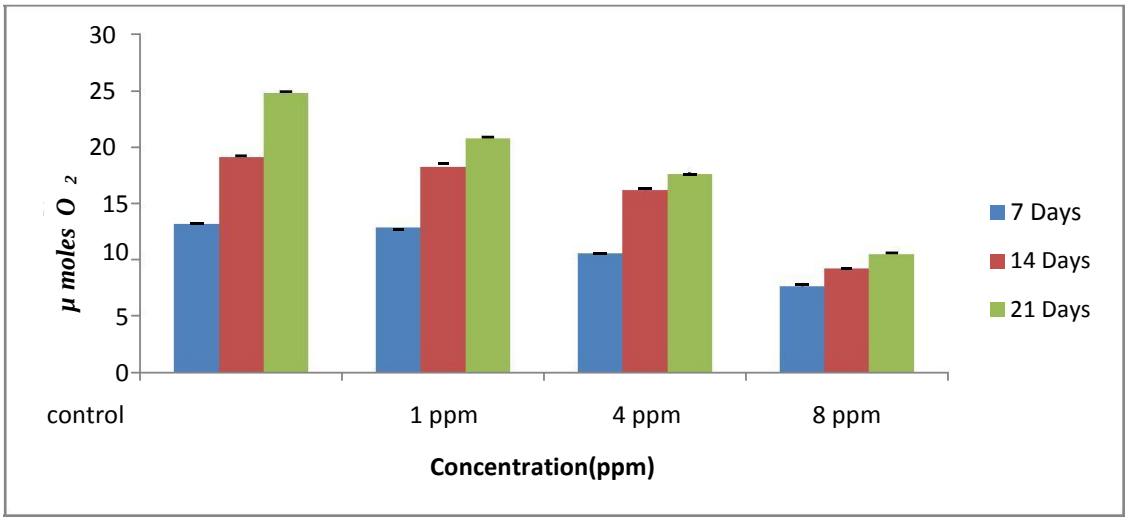

Fig. 36: Nitrate reductase activity in presence of Chlorpyrifos

Lower concentration of chlorpyrifos also enhances the activity of nitrate reductase at 1 ppm and 4 ppm. At 1 ppm percentage increase was 9.1%, 6.4% and 7.6% respectively after 7, 14 and 21 days as compared to control. At 4 ppm percentage increase was 3.1%, 1.6% and 1.7% respectively after 7, 14 and 21 days in comparison to control (Fig. 36).

Nitrate reductase activity in *Desmonostocsp.*

In case of pretilachlor, butachlor and monocrotophos there was decrease in nitrate reductase activity as the concentration of herbicide and insecticide increases (Fig. 37, 38, 39). At 1 and 4 ppm of chlorpyrifos there was stimulatory effect on the nitrate reductase activity (Fig. 40).

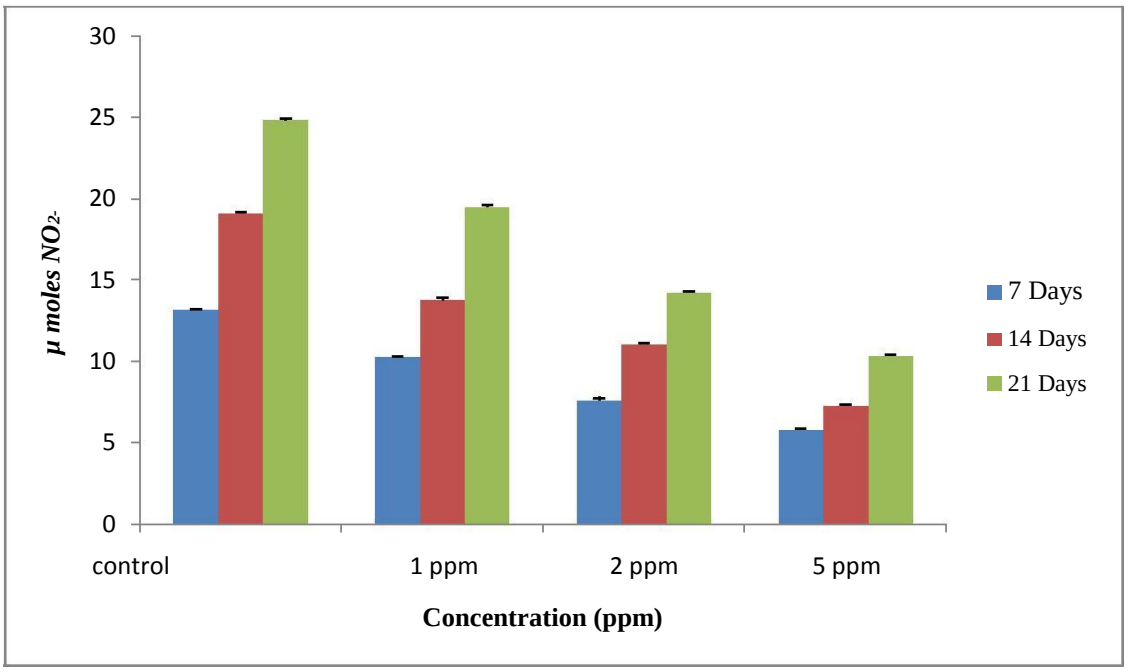

Fig. 37: Nitrate reductase activity in presence of pretilachlor

At 1 ppm decrease in nitrate reductase activity was 4%, 26% and 18% after 7, 14 and 21 days respectively. At 2 ppm decrease in activity was 14%, 36% and 45% after interval of each 14 days. At 5 ppm decrease in activity 25%, 64% and 58% after every 14 days (Fig. 37).

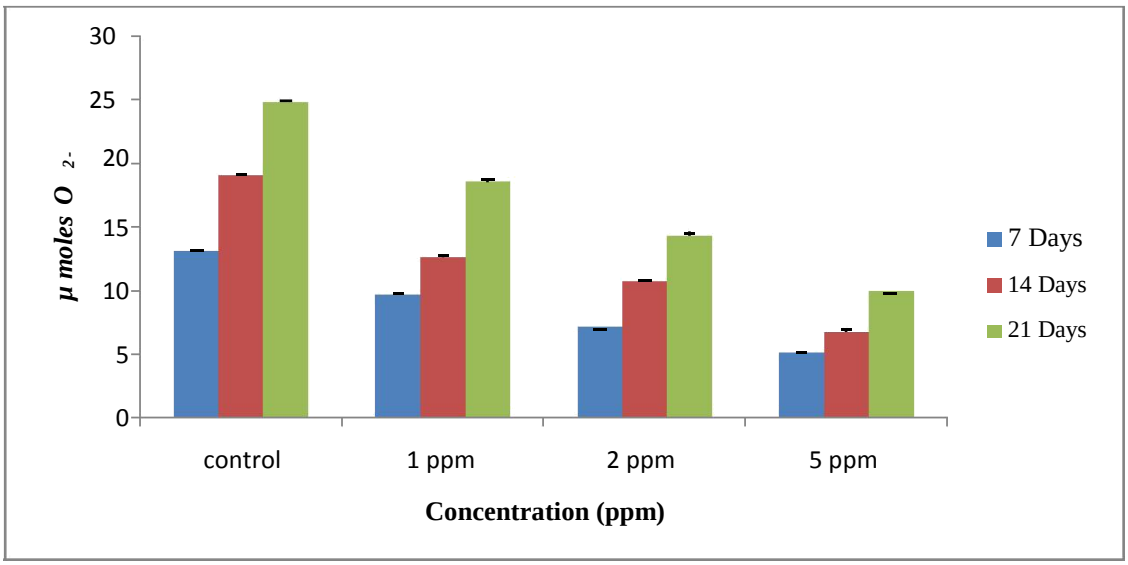

Fig. 38: Nitrate reductase activity in presence of butachlor

After 14 and 21 days percentage decrease in nitrate reductase activity was 12% at 1 ppm. At 4 ppm decrease in nitrate reductase activity was 10%, 36% and 28% after 7, 14 and 21 days. At 8 ppm decrease in activity was 22%, 55% and 57% after interval of every 14 days (Fig. 38).

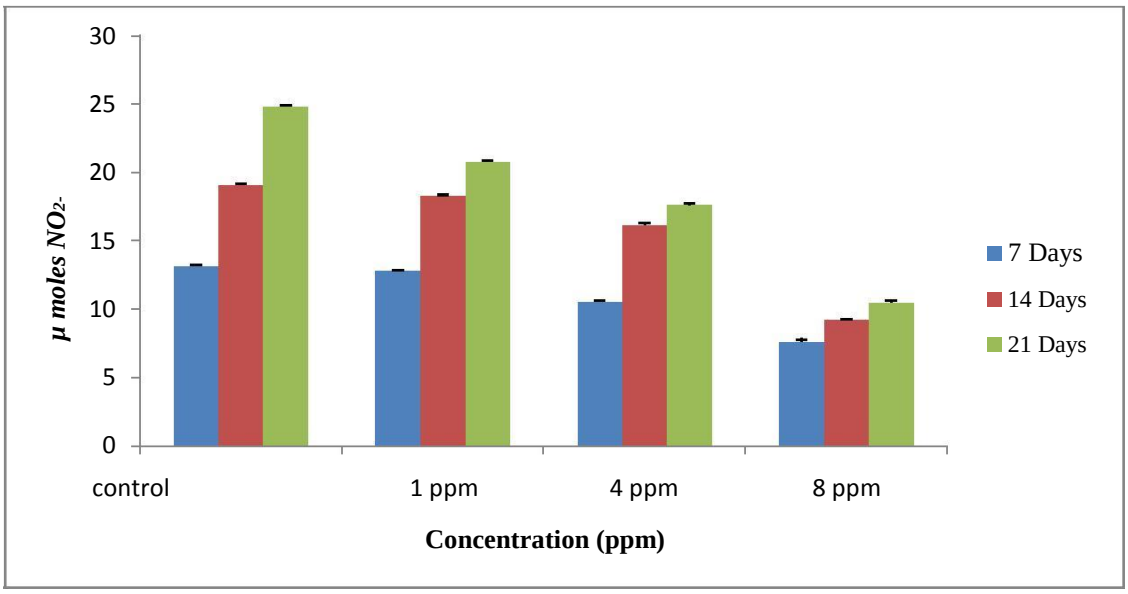

Fig. 39: Nitrate reductase activity in presence of monocrotophos

At 1 ppm there was increase in nitrate reductase activity by 5%, 1% and 2% after interval of each 14 days. At 4 ppm there was decrease in activity by 12 % and 2% after 14 and 21 days. At 8 ppm decrease in activity was 17%, 53 and 57% after 7, 14 and 21 days respectively (Fig. 39).

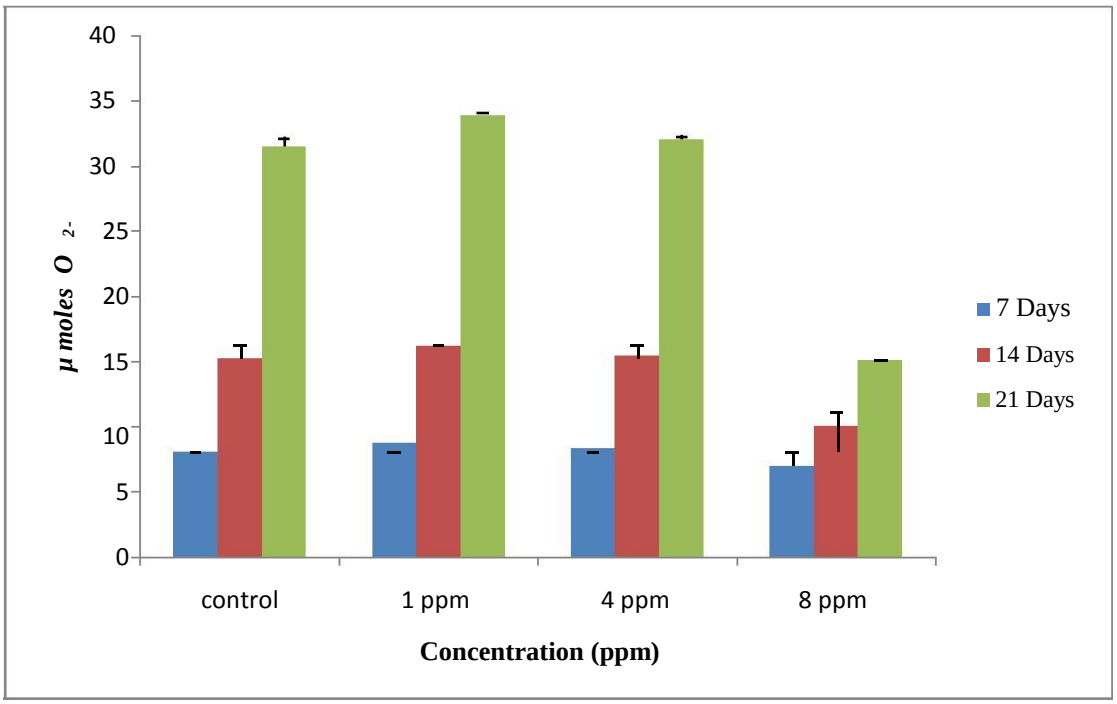

Fig. 40: Nitrate reductase activity in presence of chlorpyrifos

At 1 ppm increase in nitrate reductase activity was 9%, 6% and 7% after each interval of 14 days and at 8 ppm decrease in activity was 12%, 33% and 53% after 7, 14 and 21 days respectively (Fig. 40).

4.5 Heterocyst frequency

As the concentration of pesticide increased there was slight decrease in heterocyst frequency. Similar results were reported by many others that high concentration of pesticides decrease the heterocyst frequency and cell lysis occurs. However, at lower concentration the heterocyst frequency was similar to control with no pesticide treatment, while at 5 ppm of pretilachlor and butachlor there was decline in the heterocyst

frequency. Monocrotophos and chlorpyrifos resulted decrease in heterocyst frequency at 8 ppm. Heterocyst is the of nitrogen fixation therefore at higher concentration due to decline in heterocyst frequency total nitrogen fixation will get affected negatively. These results are comparable to the literature reported by Singh *et al*, (2014), according to which the heterocyst frequency of *Desmonostoc muscorum* decreased by 34% at 10 ppm of pretilachlor as compared to control. Inderjit and Kaushik (2010) reported that

Anabaena fertilissima when treated with propanil resulted in the leakage of protoplast from the heterocyst due to the breakage of the plasma membrane and surrounding wall.

Table 13: Heterocyst frequency of *Anabaena variabilis* and *Desmonostoc sp.* at different graded concentration of different pesticide

Pesticide	**Concentration**	***Anabaena variabilis***	***Desmonostoc sp.***
		Heterocyst frequency after 21 days (%)	**Heterocyst frequency after 21 days (%)**
No pesticide	Control	16.03	19.2
Pretilachlor (Herbicide)	1 ppm	16	18.5
	2 ppm	16.1	16
	5 ppm	14.2	15.67
Butachlor (Herbicide)	1 ppm	17	18.9
	2 ppm	16.2	17.5
	5 ppm	13.8	17
Monocrotophos (Insecticide)	1 ppm	17	19.03
	4 ppm	16.02	18.9
	8 ppm	15.3	18.3
Chlorpyrifos (Insecticide)	1 ppm	16.5	18.9
	4 ppm	16.02	18
	8 ppm	15.8	17.9

CONCLUSION

At three times higher concentration than the recommended dose of herbicides and insecticides that are commonly used, there was no inhibition in the growth of *Anabaena variabilis* and *Desmonostoc sp.* as judged by dry biomass production, chlorophyll content, Nitrate reductase activity and heterocyst frequency.

At 10 times more concentration of herbicides *Anabaena variabilis* showed inhibition of growth.

Desmonostoc sp. is able to tolerate higher concentration of herbicides (butachlor and pretilachlor) and insecticides (monocrotophos and chlorpyrifos) as compared to *Anabaena variabilis*.

Lower concentration of pesticides even shows stimulatory effect on the growth of cyanobacteria.

Very high concentrations of pesticides cause decline in heterocyst frequency, heterocyst are sight of nitrogen fixation thus affecting the nitrogen fixation.

Since at low concentration of insecticides the physiological process of organism was not adversely affected, the organism can be used in biofertilizer technology program for insecticides (monocrotophos and chloropyrifos) and herbicides (butachlor and pretilachlor) applied fields.

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