Therapeutic and Nutritional potential of *Spirulina maxima* cultivated under electromagnetic field and different light intensities in an air lift photobioreactor

Final Progress Report

Principal Investigator

Prof. GBKS. Prasad
SOS in Biotechnology
Jiwaji University, Gwalior

Submitted to

MADHYA PRADESH BIOTECHNOLOGY COUNCIL
BHOPAL

Madhya Pradesh Biotechnology Council

(1) Project Title :Therapeutic and nutritional potential of Spirulina maxima cultivated under electromagnetic field and different light intensities in airlift photobioreactor

(2) PI :Dr.GBKS Prasad
School of studies in Biotechnology
Jiwaji University Gwalior (M.P) 474011

(3) Date of Start :12.10.07

(4) Duration :3 Years

Objectives of the project

1. To study the effect of EMF of different intensities on *Spirulina* biomass production

2. To study the effect of EMF on nutritional composition of *Spirulina maxima* in terms of essential amino acid and trace element.

3. To study the effect on EMF on chlorophyll content of *Spirulina maxima*.

4. To study the influence of different light intensities (1000-10000 lx) on growth and chlorophyll content of *Spirulina maxima* in an airlift bioreactor.

5. To study the combined effect of EMF and light on phycocyanin content of *Spirulina maxima* and its antioxidant potential.

6. To evaluate the antidiabetic potential of Spirulina maxima with different biochemical and nutritional compositions grown under different EMF and /or light intensities.

List of Abbreviations

A(s)	-	Absorbance of sample
A (std)	-	Absorbance of standard
ALP	-	Airlift photobioreactor
AUC	-	Area under curve
BSA	-	Bovin serum albumin
Chl-a	-	Chlorophyll-a
DC	-	Diabetic control
DM	-	Diabetes mellitus
DO	-	Dissolved oxygen
EMF	-	Electromagnetic Field
FBG	-	Fasting blood glucose
FCR	-	Folin-Ciocalteau reagent
G	-	Gauss
GDM	-	Gestational diabetes mellitus
GLA	-	Gamma linolenic acid
GP	-	Group
IDDM	-	Insulin dependent diabetes mellitus
LA	-	Linoleic acid
NC	-	Normal control
NIDDM	-	Non-insulin dependent diabetes mellitus
MS	-	Metabolic syndrome
OD	-	Optical density
S. maxima	-	*Spirulina maxima*

LEGENDS OF FIGURES

FIG 1: EFFECT OF S. MAXIMA EXTRACT ON BLOOD GLUCOSE LEVEL IN FRUCTOSE INDUCED HYPERGLYCEMIA.

FIG 2.1: EFFECT OF S. MAXIMA EXTRACT ON TRIGLYCERIDE LEVEL IN FRUCTOSE INDUCED HYPERTRIGLYCEREDEMIA.

FIG 2.2: EFFECT OF S. MAXIMA EXTRACT ON CHOLESTEROL LEVEL IN FRUCTOSE INDUCED HYPERCHOLESTEREMIA.

FIG 2.3: EFFECT OF S. MAXIMA EXTRACT ON HDL-C, LDL AND VLDL LEVELS.

FIG 3(A): EFFECT OF S. MAXIMA EXTRACT ON SERUM GLUTAMATE PYRUVATE TRANSAMINASE (SGPT) ACTIVITY.

FIG 3 (B): EFFECT OF S. MAXIMA EXTRACT ON SERUM GLUTAMATE OXALOACETATE TRANSAMINASE

FIG 4.1: EFFECT OF S. MAXIMA EXTRACT ON REDUCED GLUTATHIONE (GSH) LEVEL.

FIG 4.2: EFFECT OF S. MAXIMA EXTRACT ON THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) LEVEL

FIG 4.3: EFFECT OF S. MAXIMA EXTRACT ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY.

FIG 4.4: EFFECT OF S. MAXIMA EXTRACT ON CATALASE (CAT) ACTIVITY.

FIG 5 SCHEMATIC DESIGN OF AIRLIFT PHOTOBIOREACTOR

FIG:6.1: CULTURE DENSITY OF S. MAXIMA IN AIRLIFT PHOTOBIOREACTOR UNDER ELECTROMAGNETIC EXPOSURES

FIG :6.2 THE SPECIFIC GROWTH RATE OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER ELECTROMAGNETIC EXPOSURES

FIG 6.3 : CHLOROPHYLL- A CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

FIG : 6.4 CAROTENOID CONTENTOF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

FIG : 6.5 PHYCOCYANIN CONTENTOF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

FIG : 6.6 ALLOPHYCOCYANIN CONTENTOF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.
FIG : 6.7 PHYCOERYTHRIN CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.
FIG :6.8 TOTAL CARBOHYDRATE CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.
FIG : 6.9 TOTAL PROTEIN CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.
FIG 7.0 FTIR FINGERPRINT OF *S.MAXIMA* EXPOSED TO EMF
FIG : 8.0 HPLC OF AMINOACID STANDARDS
FIG :9.0 AMINOACID FINGER PRINT OF S. MAXIMA EXPOSED TO CONTROL
FIG:9.1 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 1G EMF.
FIG: 9.2 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 400 G EMF
FIG 10 TRANSMISSION ELECTRON MICROGRAPH OF S. MAXIMA EXPOSED TO EMF
FIG 11 TRANSMISSION ELECTRON MICROGRAPH OF S. MAXIMA EXPOSED TO EMF
FIGURE (12) - FASTING BLOOD GLUCOSE LEVELS OF RATS KEPT ON 72% FRUCTOSE RICH DIET
FIGURE (13) - S. MAXIMA ADMINISTRATION IN FASTED NORMAL ANIMALS
FIGURE (14) - AREA UNDER CURVE GRAPH OF NORMAL FASTING ANIMALS.
FIGURE (15) - S. MAXIMA ADMINISTRATION IN DIABETIC FASTED ANIMALS.
FIGURE (16) - AREA UNDER CURVE GRAPH OF DIABETIC FASTING ANIMALS
FIGURE (17) – S. MAXIMA ADMINISTRATION IN NON-FASTED NORMAL ANIMALS.
FIGURE (18) - AREA UNDER CURVE GRAPH OF NORMAL NON-FASTING ANIMALS.
FIGURE (19) – S. MAXIMA ADMINISTRATION IN DIABETIC NON-FASTED ANIMALS.

FIGURE (20) - AREA UNDER CURVE GRAPH OF DIABETIC NON-FASTING ANIMALS.
FIGURE (21)- GROWTH RATE OF S. MAXIMA EXPOSED TO HIGH LIGHT INTENSITY
FIG :22 CHLOROPHYLL-A CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG :23 CAROTENOID CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG :24 TOTAL PROTEIN CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG :25 CARBOHYDRATE CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG :26 PHYCOCYANIN CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG :26 ALLOPHYCOCYANIN CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG :26 PHYCOERYTHRIN CONTENT OF S. MAXIMA CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE AND HIGH LIGHT INTENSITY
FIG: 27 MINERAL ANLYSIS OF S. MAXIMA EXPOSED TO EMF
FIG 28 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO CONTROL
FIG 29 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 1G EMF AND HIGH LIGHT INTENSITY.
FIG 30 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 400 G EMF AND HIGH LIGHT INTENSITY.
FIG- 31 SCHEMATIC DIAGRAM TO SHOW THE FACTOR ANALYSIS

FIG -32 : PROTEIN, CARBOHYDRATE AND LIPID CONTENT OF *S.MAXIMA* CULTURED UNDER DIFFERENT SODIUM NITRATE CONCENTRATIONS.

FIG -33 : NON ENZYMATIC ANTIOXIDANTS OF SPIRULINA MAXIMA CULTURED UNDER DIFFERENT SODIUM NITRATE CONCENTRATIONS.

FIG -34 : BIOMASS AND SPECIFIC GROWTH RATE OF SPIRULINA MAXIMA CULTURED UNDER DIFFERENT SODIUM NITRATE CONCENTRATIONS.

FIG -35 : PHYCOBILIPROTEIN CONTENT OF SPIRULINA MAXIMA CULTURED UNDER DIFFERENT SODIUM NITRATE CONCENTRATIONS.

LIST OF TABLES

TABLE-1: AMINO ACID STANDARDS WITH RETENTION TIME

TABLE -2 :EXOGENOUS AMINO ACID COMPOSITION OF S. MAXIMA

TABLE -3 :ENDOGENOUS AMINO ACID COMPOSITION OF S. MAXIMA

TABLE- 4: PERCENTAGE INCREASE IN CONCENTRATIONS OF DIFFERENT METABOLTES WITH RESPECT TO SODIUM NITRATE CONCENTRATIONS.

TABLE- 5: PRINCIPAL COMPONENT ANALYSIS: OF S. MAXIMA CULTURED IN DIFFERENT CONCENTRATIONS OF SODIUM NITRATE.

TABLE:6(a): CHLOROPHYLL-A, CAROTENOID AND PHYCOBILIPROTEIN CONTENTS OF S. MAXIMA GROWN IN ZARROUK'S MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF SODIUM NITRATE.

TABLE 6 (b): PROTEIN, CARBOHYDRATE, ASCORBIC ACID, PHENOL CONTENTS OF S. MAXIMA GROWN IN ZARROUK'S MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF SODIUM NITRATE.

Contents

Chapter 1: Antidiabetic potential of Spirulina *maxima* page**:** 12 - 42

Introduction - 12

Review of literature -13

Experimental methods -16

Results -25

Chapter 2 : ***EFFECT OF EMF ON THE METABOLITE CONTENT AND THERRAPWUTIC PROPERTY OF S.MAXIMA*** page **:** 43 - 42

Introduction -43

Review of literature -47

Experimental methods -52

Results -55

Discussion -95

Conclusion -96

Summary of the project 97

Chapter 3: ***Biomass production of S.maxima by modulating the compositions of nutrient supplementation*** page **:**98 - 107

Introduction - 98

Review of literature

Experimental methods 108

Results 117

Discussion 120

Conclusions 122

Summary of the project 123

References 121

CHAPTER 1

Antidiabetic Potential of *S. maxima*

Introduction

Diabetes mellitus is a chronic degenerative disorder with increasing morbidity and mortality, characterized by hyperglycemia and often associated with hyperlipidemia and oxidative stress. Diabetes is the metabolic disorder, which can affects nearly every organ system in the body and is generally viewed as a clinical syndrome with variable phenotypic expression rather than a single disorder with a specific etiology. It is often associated with hyperlipidemia and hypertension (Pradeepa et al., 2002).Several clinical studies indicate that patients with type 2 diabetes are also subjected to chronic oxidative stress (Baynes et al., 1996).

Emerging evidence from recent epidemiological and biochemical studies clearly suggest that exposure to high fructose, lead to rapid stimulation of lipogenesis and triglyceride accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance (Basciano et al., 2005). Recent studies reveal that fructose feeding promotes oxidative damage and exert detrimental effects by reducing antioxidant defenses, and increasing generation of free radicals (Faure et al., 1997).Apart from currently available therapeutic options, many herbal medicines appeared highly promising for the treatment of diabetic syndrome[5]. *S. maxima*, a filamentous alga, is a cyanobacterium belongs to the family Oscillatoriaceae, which blooms well in alkaline waters[6]. This cyanobacterium grows naturally in warm climate countries and is consumed by humans. It is characterized by high protein content (60-70%) and also contains considerable levels of chlorophyll, carotenoids, phycocyanin and vitamins (Kulshreshta et al., 2008). Besides the

organism's pivotal role in the treatment of protein malnutrition disorders, *S. maxima* does bring down body weight in obese humans (Becker et al., 1986) and plasma cholesterol level (Itawa et al., 1990). *S. maxima* prevented fatty liver in rats after the administration of fructose rich purified diet (Gonzalez et al., 1993) or by carbon tetrachloride treatment. Furthermore, *S. maxima* has been shown to decrease vascular tone of aortic rings (Paredes-Carbajal et al., 1997).

Review of literature

Spirulina is a microscopic and filamentous cyanobacterium that belongs to family Oscillatoriaceae and has a long history of use as food and food supplement. Its name derives from the spiral or helical nature of its filaments. It was used as food in Mexico during the Aztec civilization some 400 years ago. It is still being used as food by the Kanembu tribe in the lake Chad area of the Republic of Chad where it is sold as dried bread called "dihe" (Abdulqader et al., 2000). *Spirulina* has been produced commercially for the last 20 years for food and specially feeds (Belay et al., 1994; 1996;1997). Habitats for *Spirulina* include the Pacific Ocean near Japan and Hawaii, and large freshwater lakes, including Lake Chad in Africa, Klamath Lake in North America, Lake Texcoco in Mexico, and Lake Titikaka in South America. The current use of this resource has three precedents: tradition, scientific and technological development, and the so-called, "green tendency" (Henrikson; 1994). From 1970, the nutritional and medicinal studies on *Spirulina* have proliferated (Hayashi et al., 1996). In 1970, the German Federal Republic supported investigations on human consumption of *Spirulina* in India, Thailand and Peru. In the Asian countries, the production was focussed on nutritious support for the undernourished population. The supplementation of meals with *Spirulina* could be a solid and cost-

effective option to provide to the most vulnerable populations a solid basis of physical and mental health. Although this ancient alga has been consumed for centuries by traditional people, it was only rediscovered by scientists 30 years ago.

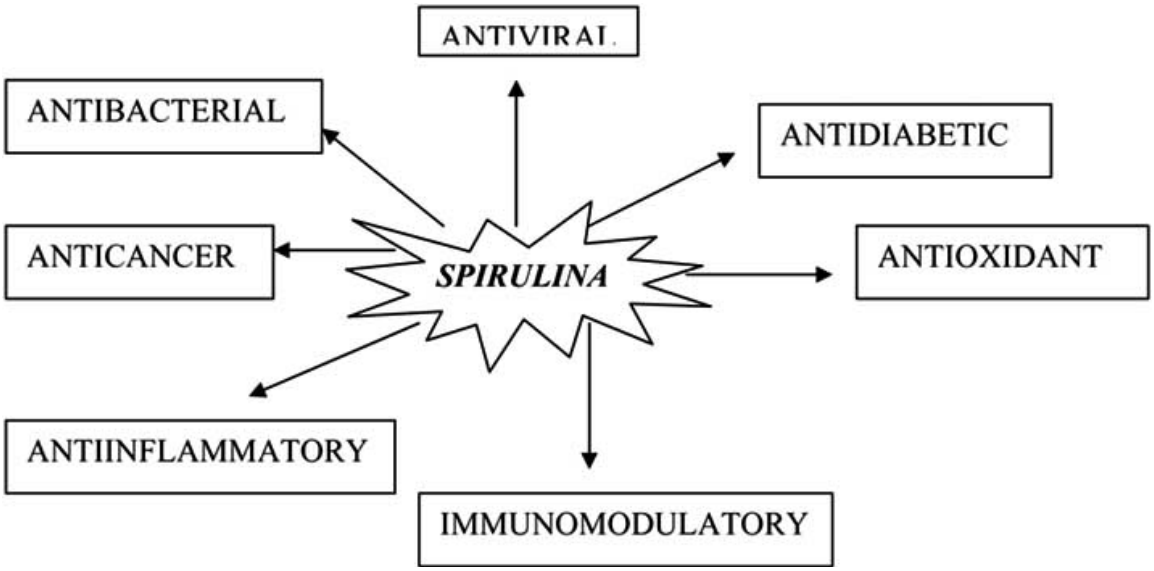


Fig. (1). Applications of *spirulina* in health care.

ANTI-OXIDANT PROPERTIES OF *SPIRULINA*

Several studies have demonstrated that *Spirulina* possess significant antioxidant activity both *in vitro* and *in vivo*. (Miranda et al., 1998) studied the antioxidant activity of carotenoids, phenolics and tocopherols extracted from *S. maxima* and found that the phenolic compounds responsible for the antioxidant properties of the *S. maxima* extracts were organic acids (caffeic, chlorogenic, quimic, salicylic, synaptic and trans-cinnnamic) which acted individually and synergistically while (Estrada et al., 2001) demonstrated the antioxidant activity of the phycobiliproteins, phycocyanin and allophycocyanin present in *Spirulina* biomass. (Manoj et al 1992) reported that the alcohol extract of *Spirulina* inhibited lipid peroxidation more significantly (65%) than the chemical

antioxidants like α-tocopherol (35%), butylated hydroxyl anisol (45%) and β-carotene (48%). The water extract of *Spirulina* is also shown to have more antioxidant effect (76%) than gallic acid (54%) and chlorogenic acid (56%). Phycocyanin also inhibited liver microsomal lipid peroxidation. (Zhi-gang et al., 1997) studied the antioxidant effects of two fractions of a hot water extract of *Spirulina* using three systems that generate superoxide, lipid, and hydroxyl radicals. Both fractions showed significant capacity to scavenge hydroxyl radicals (the most highly reactive oxygen radical) but no effect on superoxide radicals. One fraction had significant activity in scavenging lipid radicals at low concentrations

ANTI-DIABETIC PROPERTIES OF *SPIRULINA*

Spirulina has been shown to possess antihyperglycemic and antihyperlipidemic properties in experimental models. In patients with type-2 diabetes mellitus, *Spirulina* diet lowered fasting blood glucose, postprandial glucose and reduction in the glycosylated haemoglobin (HbA-1c) (Khan et al., 2005). The aqueous extract of

S. maxima is very effective in alleviating the abnormalities of carbohydrate and lipid metabolisms induced by excess fructose in Wistar rats. Treatment with *Spirulina* in diabetic rats increased the hexokinase activity and decreased the glucose-6- phosphatase activity. *Spirulina* has a beneficial effect on plasma insulin and C-peptide (Layam et al., 2006). In a study measuring the effect of blue green algae on glucose levels in diabetic rats & mice, the water soluble fraction is found to be effective in lowering the serum glucose level at fasting as well as on glucose loading (Rodriguez-Hernandez et al., 2001; Takai et al., 1991). *S. maxima* exhibited hypolipidemic effects, especially on triacylglycerols (TAG) and the LDLCholesterol [78] and prevented dyslipidemia induced by carbon tetrachloride (Torres Duran et al., 1998). The elevation of total cholesterol, LDL and VLDL cholesterol and

phospholipids in the serum was reduced significantly when the experimental high cholesterol diet was supplemented with 16% *Spirulina* (Kato et al., 1984). The fall in HDL cholesterol caused by the high cholesterol diet is also prevented in mice fed with *Spirulina*. Adipohepatosis induced by a high fat and high cholesterol diet is also reduced rapidly when the mice are shifted from the high fat, high cholesterol diet to a basal medium supplemented with *Spirulina.* Liver levels of triglycerides and phospholipids responded significantly in rats fed a diet supplemented with 5% *Spirulina* and either 60% glucose or 60% fructose (De Rivera et al., 1993). Hence the study was mainly focused on the antioxidant, antihyperglycemic and antioxidant property of *Spirulina maxima* against fructose induced diabetes and other metabolic abnormalities in Wistar rats.

Experimental Design

The care and maintenance of animals were as per the approved guidelines of the "Committee for the purpose of control and Supervision of Experiment on Animals." (CPCSEA, India). Animals were divided into seven groups and treated as below.

Group I (n=5) : Healthy rats (Normal control)

Group II (n=5) : Hyperglycemic rats without any treatments (Diabetic control, DC)

Group III (n=5): Hyperglycemic rats administered with 5% *S.maxima* extract (SM) once daily for following 30 days (Diabetic rats treated with 5% SM) .

Group IV (n=5): Hyperglycemic rats co-administered with 10% fructose solution continuously (CF) and 5% *S. maxima* extract once daily for following 30 days (Diabetic rats treated with 5% SM and 10% CF concurrently).

Group V (n=5): Hyperglycemic rats administered with 10% *S.maxima* extract once daily for following 30 days (Diabetic rats treated with 10% SM).

Group VI (n=5): Hyperglycemic rats co-administered with 10% fructose solution and 10% *S. maxima* extract once daily for following 30 days (Diabetic rats treated with 10%SM and 10% CF concurrently).

Group VII (n=5): Hyperglycemic rats administered with metformin (500 mg/kg) once daily for following 30 days (Metformin).

Preparation and administration of S. maxima extract

The dried *S. maxima* powder was suspended in distilled water to achieve a concentration of 5% and 10% (i.e. 5 and 10 gm of *Spirulina* powder was suspended in 100 ml of distilled water). The homogenous suspension of *S. maxima* was prepared and administered orally with a dose of 1.0 ml/day. The doses were administered once a day to experimental rats with the help of intragastric tube (cannula).

Animals

Randomly bred healthy male rats (*Rattus norvegicus*; Wistar strain; age- 10-14 weeks; weight-160-200gm) or BALB/c mice (weight-20-25gm) were obtained from the animal facility division of Defense Research & Development Establishment, Gwalior (M.P). Before and during the experiment, the animals were maintained on standard pellet diet (Amrut Ltd., India) and water *ad libitum*. After randomization into various groups and before initiation of experiment, the animals were acclimatized for a period of 7 days to laboratory conditions [Temperature (25± 2°C) and 12 hour light/dark cycle]. The study protocol was approved by Institutional Animal Ethics Committee (IAEC). The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose and Supervision of Experiments on Animals (CPSEA).

Processing of blood samples - The animals were kept on 6-8 hrs fasting and then the blood samples were collected from retro orbital plexus of rat eyes under mild ether anesthesia through heparinized capillaries into heparinized vials once before feeding with fructose (i.e. 0 day) and at 10 day intervals (i.e. 10, 20, 30, 40, 50 and 60 days) upto 60 days, plasma was separated in a cooling centrifuge at 2000rpmx10minx4˚C. The plasma was aliquoted and used for analyses of glucose, triglyceride and cholesterol, remaining plasma sample was frozen at -20˚C and later assayed for HDL (high density lipoprotein) cholesterol, LDL (low density lipoprotein), VLDL (very low density lipoprotein) and serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT).The liver markers (SGPT and SGOT) and antioxidant markers such as reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) were analyzed before and after induction of diabetes and after *Spirulina* therapy (i.e. 0, 30 and 60 days).

A part of whole blood sample (0.2 ml) was used for estimation of reduced glutathione (GSH) and remaining sample was used for preparation of haemolysate for estimation of CAT, SOD and TBARS. All estimations were carried out immediately after collecting the blood samples. The biochemical analyses of the separated plasma were carried out in detailed in article 3.4.3.4. The blood samples were analyzed to study the antioxidant markers as detailed in article 3.4.3.5. The protein tests were carried out on haemolysate and the procedure adopted is presented in article 3.4.3.6.

Biochemical Analyses of plasma

The chemical reactions and procedures involved for the estimations of various biochemical parameters are presented herein.

Estimation of Blood glucose (GOD-POD method) (Trinder, 1969) - Glucose by the enzymatic reaction of Glucose oxidase (GOD) gets converted to Gluconic acid and hydrogen peroxide (H_2O_2). In the subsequent enzymatic reaction this is converted into red color quinoneimine in a reaction with aminoantipyrine and phenol catalyzed by peroxidase (POD). The reactions involved and procedure for estimation of blood glucose are as under.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4\ \text{Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red Quinoneimine} + H_2O$$

The reaction mixture contained 1.0 ml of working reagent 2 (Appendix 2), 0.01 ml of glucose standard (reagent 1, Appendix 2) or 0.01 ml of plasma. The contents were vortexed and incubated for 15 minutes in RT. The absorbance was read against the buffer as blank at 505 nm. The values are expressed in mg/dl.

Estimation of Triglycerides (GPO-POD method) (Wood et al., 1998) - Triglyceride (a form of lipid), by the action of Lipase, hydrolyze to glycerol and fatty acid. Glycerol by the enzymatic reaction of Glycerol Kinase (GK) gets converted into Glycerol-3-phosphate. In the subsequent enzymatic oxidation by Glycerol-3-phosphate oxidase (GPO), H_2O_2 is formed. This is converted into colored chinonimine in a reaction with aminoantipyrine and chlorophenol catalyzed by peroxidase (POD). The reactions involved and procedure for estimation of triglyceride are as follows:

$$\text{Triglyceride} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydro-acetone- phosphate} + H_2O_2$$

$$2H_2O_2 + 4\ \text{aminoantipyrine} + 4\ \text{chlorophenol} \xrightarrow{\text{POD}} \text{Chinonimine} + 4H_2O$$

The reaction mixture contained 1.0 ml of working reagent 1 (Appendix 2), 0.01 ml of standard (reagent 2, Appendix 2) or 0.01 ml of plasma. The contents were vortexed and incubated for 20 minutes in RT. The absorbance was read against the buffer as blank at 546 nm within 60 minutes. The values are expressed in mg/dl.

Estimation of Cholesterol (CHOD-PAP method) (Deeg et al., 1983) -Cholesterol and its esters are released from lipoprotein by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed. This

is converted into a colored quinonimine in a reaction with p-aminophenazone (PAP) and phenol catalyzed by peroxidase. The procedure for estimation of cholesterol is as follows. The reaction mixture contained 1.0 ml of working reagent 1(Appendix 2) 0.01 ml of standard (reagent 2, Appendix 2) or 0.01 ml of plasma. The contents were vortexed and incubated for 20 minutes in RT. The absorbance was read against the buffer as blank at 546 nm within 45 minutes. The values are expressed in mg/dl.

Estimation of HDL-Cholesterol (Phosphotungstate method) (Lopes-virella, 1977) - Chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoprotein) fractions in plasma are separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, is assayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen 4-Aminoantipyrine/phenol. The procedure for determination of HDL-Cholesterol is presented below.

To 0.2 ml of precipitating reagent (reagent 2, Appendix 2), 0.2 ml of sample (plasma) is added, mixed and centrifuged at 3500rpmx10minxRT. The clear supernatant was separated immediately for the HDL-cholesterol estimation. The reaction mixture contained 1.0 ml of reconstituted reagent (Appendix 2), 0.02 ml of cholesterol standard or 0.02 ml of separated supernatant as test sample. The contents were vortexed and incubated for 10 minutes in RT. The absorbance was read against the buffer as blank at 520 nm. The values are expressed in mg/dl.

If the value of the Triglycerides (TG) and total cholesterol (TC) is known, VLDL and LDL-Cholesterol can be calculated based on Friedewald's equation as under.

VLDL (mg/dl) = TG/5

LDL-C (mg/dl) = TC – (VLDL+ HDL-C)

Estimation of ALT/SGPT (Reitman and Frankel (1957) – The reactions involved and procedure for the determination of Alanine amino transferase (AST) also referred to as Serum Glutamate Pyruvate Transaminase (SGPT) are presented.

The SGPT (ALT) catalyses the following reaction.

α- Ketoglutarate + L- Alanine ⟶ L-Glutamate + Pyruvate

Pyruvate so formed is coupled with 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gives the brown color in alkaline medium and this can be measured colorimetrically. The assay system containing 0.25 ml of substrate solution (reagent 1, Appendix 2) and 0.05 ml plasma was incubated for 30 minutes at 37°C in water bath. To it, 0.25 ml of chromogen solution (reagent 2, Appendix 2) was added, mixed and allowed to stand for 20 minutes at room temperature. 2.5 ml of NaOH (reagent 3, Appendix 2) was added to above mixture and the optical density was read after 5 minutes against blank at 505 nm, running the controls parallely. The enzyme activity is expressed in units/L.

Estimation of AST/SGOT (Reitman and Frankel (1957) – The reactions involved and the procedure for the determination of Aspartate amino transferase (AST) also known as Serum Glutamate Oxaloacetate Transaminase (SGOT) are included herein.

The SGOT (AST) catalyses the following reaction.

α- Ketoglutarate + L- Aspartate ⟶ L-Glutamate + Oxaloacetate

Oxaloacetate so formed is coupled with 2, 4-Dinitrophenyl hydrazine

(2, 4-DNPH) to give the corresponding hydrazone, which gives the brown color in alkaline medium and this can be measured colorimetrically.

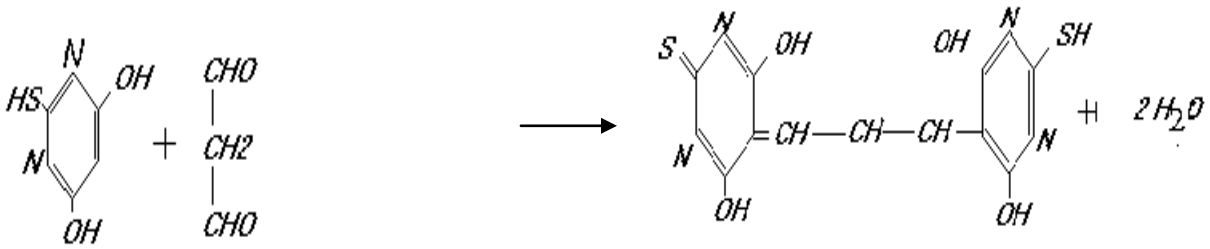
The assay system containing 0.25 ml of substrate solution (reagent 1, Appendix 2) and 0.05 ml of plasma was incubated for 60 minutes at 37°C in water bath. To it 0.25 ml of chromogen solution (reagent 2, Appendix 2) was added and the mix was allowed to stand for 20 minutes at room temperature. 2.5 ml of NaOH (reagent 3, Appendix 2) was added to above mixture and the optical density was read after 5 minutes against blank at 505 nm, running the controls parallely. The enzyme activity is expressed in units/L.

Estimation of antioxidant markers in blood.

The procedure for estimation of antioxidant markers in the blood is included in this article. For the preparation of packed cell volume (PCV) or haemolysate, the plasma and the buffy coat were removed from whole blood cells by centrifugation at 2000 rpmx10minx4°C. The red cells were washed thrice with chilled normal saline (0.9%) to get the PCV. From this PCV, haemolysate(s) for further estimations were prepared as follows. For the determination of CAT and TBARS, the haemolysate was prepared by making 5% PCV solution in chilled distilled water i.e. 1.9 ml of chilled distilled water to 0.1 ml of PCV suspension. For the determination of SOD, the remaining red cells were haemolysed by adding distilled water. The lipids were removed by using chloroform-ethanol extraction. For this, the haemolysate was diluted four times with ice-cold distilled water. To 4 ml haemolysate, 1ml of ethanol and 0.6ml of chloroform were added sequentially with continuous shaking for 1 min. The preparation was subjected to centrifugation at 3000rpmx10minx4°C. The supernatant was used for the estimation of SOD. The haemolysate(s) so prepared were used for the following anti-oxidant tests.

TBARS (Ohkawa et al., 1979) **–** The reactions involved and procedure for the determination of Thiobarbituric acid reactive substance (TBARS) are detailed as follows.

The Malonaldehyde (MDA), a decomposition product of lipid hydro peroxides is used as an indicator of oxidative damage to cells and tissues. In this method, the test sample heated with thiobarbituric acid (TBA) under acidic conditions develops pink color of various compounds that react with TBA. The spectrophotometric test is conducted at 532 nm. A small amount of MDA is produced during per oxidation which reacts with the TBA in this test to generate a colored product, by the following reaction.



TBA MDA Colored Product

In acid solution, the product absorbs light at 532 nm and is readily extractable into organic solvents such as n-butanol. For the test, 0.4 ml of 5% RBC haemolysate was incubated with 0.1 ml of 8.1% sodium dodecyl sulphate (SDS) (w/v) for 10 minutes followed by addition of 20% acetic acid (pH 3.5). The reaction mixture was incubated along with 0.6% thiobarbituric acid (w/v) for 1 hour in boiling water bath. The pink color chromogen so formed was extracted with butanol-pyridine (15:1) solution and read at 532 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56 $x10^{-5}$ mole/mm and the values are expressed as nmole/mg protein.

***SOD (Winterbourn et al., 1975*) –** The reaction/principle involved and the procedure for the determination of superoxide dismutase (SOD) are presented. The SOD catalyses the breakdown of the superoxide free radical (O_2^-) according to the reaction given below:

$$2O_2^- + 2H^+ \longrightarrow O_2^- + H_2O_2$$

This method is based on the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazan complex. Acetic acid was used to arrest the formazan formation. The colour intensity of the chromogen was measured at 560 nm.

For the determination of SOD, the RBCs were washed with normal saline and after haemolysis chloroform-ethanol extraction was performed to elute the enzyme. To 0.2 ml of blood, 1.2 ml of sodium pyrophosphate buffer (0.052mM, pH 7.0), 0.1 ml of phenazine methosulphate (180 μM) and 0.3 ml of nitro blue tetrazolium (300 μM) were added and the volume was adjusted to 1.8 ml with distilled water. Finally, 0.2 ml of NADH (780 μM) was added and the tubes were incubated at 37^0C for 90 seconds. The reaction was stopped precisely by the addition of 1.0 ml of glacial acetic acid and the tubes were read at 560 nm after 10 minutes. Controls without the supernatant were also run in parallel. The SOD activity is expressed in units/min/mg protein.

Catalase (Sinha, 1972) - This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of

perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 560-630 nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase activity in blood was assayed by the following procedure. 5% RBC haemolysate was incubated with 0.5 ml of H_2O_2 (0.2 M) at 37^oC for 90 seconds precisely, in the presence of 0.01 M phosphate buffer at pH 7.4 (Appendix 2). The reaction was stopped with addition of 5% dichromate solution. The samples were further incubated at 100^oC for 15 min in boiling water bath. Amount of H_2O_2 consumed was determined by recording absorbance of solution at 570 nm and the activity is expressed in μmole/min/mg protein.

GSH (Ellman, 1959) - This method is based on the fact that DTNB {5,5- dithio bis (2-nitro benzoic acid)} is reduced by sulfydryl group (SH), present in reduced glutathione to form one mole of 2-nitro -5- mercaptobenzoic acid per mole of SH. The reaction is as follows.The nitromercaptobenzoic acid anion has an intense yellow colour and is used to measure SH groups. Development of maximum colour intensity occurred immediately after the addition of DTNB but absorbance decreases rapidly with time.

The estimation of GSH in blood was carried by the method given by Ellman (1959) originally described by and modified by Jollow et al. (1974). To 0.2 ml of whole blood 1.8 ml of distilled water was added and the mix incubated for 10 minutes at 37^oC for complete haemolysis. To the haemolysate, 3 ml of 4% sulphosalycylic acid was added and centrifuged at 2500rpm x15minx4^0C. The supernatant (0.2 ml) was mixed with 0.4 ml of 10 mM DTNB and 1.0 ml phosphate buffer (0.1M, pH 7.4) (Appendix 2). The absorbance was measured at 412 nm. The GSH levels are expressed in mg/ml.

Estimation of Total Protein (Lowry et al., 1951)

Protein reacts with the Folin-Ciocalteau reagent to give a coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein as in the biuret test and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The

intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

To determine the total protein, 0.2 ml of the haemolysate and equal amount of 10% TCA were mixed and kept overnight. This results in the precipitation of protein. The mixture was centrifuged at 2000rpmx10minxRT and the pellets obtained were dissolved in 0.1N NaOH. The standard protein solution was prepared using bovine serum albumin (BSA) of concentration 2mg/ml. The procedure for determination is already detailed in section 3.4.1.5.

Statistical Analysis

The data obtained from the experiments were analyzed using one-way ANOVA (Fisher-LSD and Bonferroni t-test) employing Sigma Stat, statistical software, version 1.0 (Jandal corporation, USA).The values were tested for significance at $P<0.001$, $P<0.05$.

Results

Anti-diabetic and anti-oxidative effects of *S. maxima*

This investigation explored the anti-hyperglycemic, anti-hyperlipidemic and antioxidant functions of *S. maxima* grown in standard Zarrouk's medium in Wistar rat model. The diabetes was induced by feeding 10% fructose solution for 30 days. The fructose feeding was stopped at 30 days in four groups while in other two groups it was continued along with *S. maxima* therapy for another 30 days. To these hyperglycemic rats, 5% or 10% *S. maxima* extract was administered orally every day for 30 days. The body weight, blood glucose, lipid profile (triglyceride, cholesterol, HDL-C, LDL and VLDL), liver function markers (SGPT and SGOT) and antioxidant markers (GSH, TBARS, SOD and CAT) in blood were assessed before and after diabetes induction, and at the end of 30 days of *S. maxima* therapy.The basal levels of blood sugar, triglyceride, cholesterol, HDL-C (high density lipoprotein-cholesterol), LDL (low density lipoprotein) and VLDL (very low density lipoprotein) in animals of normal control group were maintained at constant level throughout the experimental period.

Effect of *S. maxima* extract on blood glucose level in fructose induced hyperglycemia.

The variation of blood glucose level with number of days is plotted The graph shows the blood glucose level along with the fructose administration and *S. maxima* and metformin therapies. It is observed that the blood glucose level is maximum at 30^{th} day when fructose administration was stopped in four groups while it was continued in two groups for another 30 days (i.e. upto 60 days), except in case normal control group in which it decline. A sharp and significant reduction in blood glucose level is obtained on initiation of *S. maxima* therapy and this continued to decline till the period of study i.e. 60 days. A significant increase in blood glucose level was recorded following administration of 10% fructose in drinking water for 30 days. The blood glucose levels increased by about 46% within 10 days of fructose administration and at the end of 30 days, the increase was 98% ($P<0.001$). The effect of 30 days continuous oral administration of *S. maxima* extract on blood glucose level was studied at two concentrations viz., 5% and 10%. The animals fed with *S. maxima* extract from 30^{th} day onwards showed in all the groups studied herein.

5% S. maxima extract

At a dose of 5% *S. maxima*, the reduction in blood glucose levels observed was 59% ($P<0.001$) at the end of the 30 days therapy in the group in which fructose was stopped at 30 days. In the continuous fructose fed rats group, the reduction in blood glucose level was 45.60%. The effect of 5% *S. maxima* extract on normal group was not studied.

10% S. maxima extract

The administration of 10% *S. maxima* extract to normal control rats showed the marginal changes in the blood glucose level by 7% at the end of 30 days therapy. The hyperglycemic rats fed with fructose upto 30 days, when treated with 10% *S. maxima* showed decrease in blood glucose level by 54% ($P<0.001$). In continuous fructose fed rats, 10% *S. maxima* reduced the blood glucose level by 48.24% at the end of the 30 days therapy. When the hyperglycemic rats were treated with metformin (500mg/kg), the blood glucose level was reduced by 45.53% after 30 days of the therapy. The reduction in blood glucose achieved by *S. maxima* was better than that of metformin.

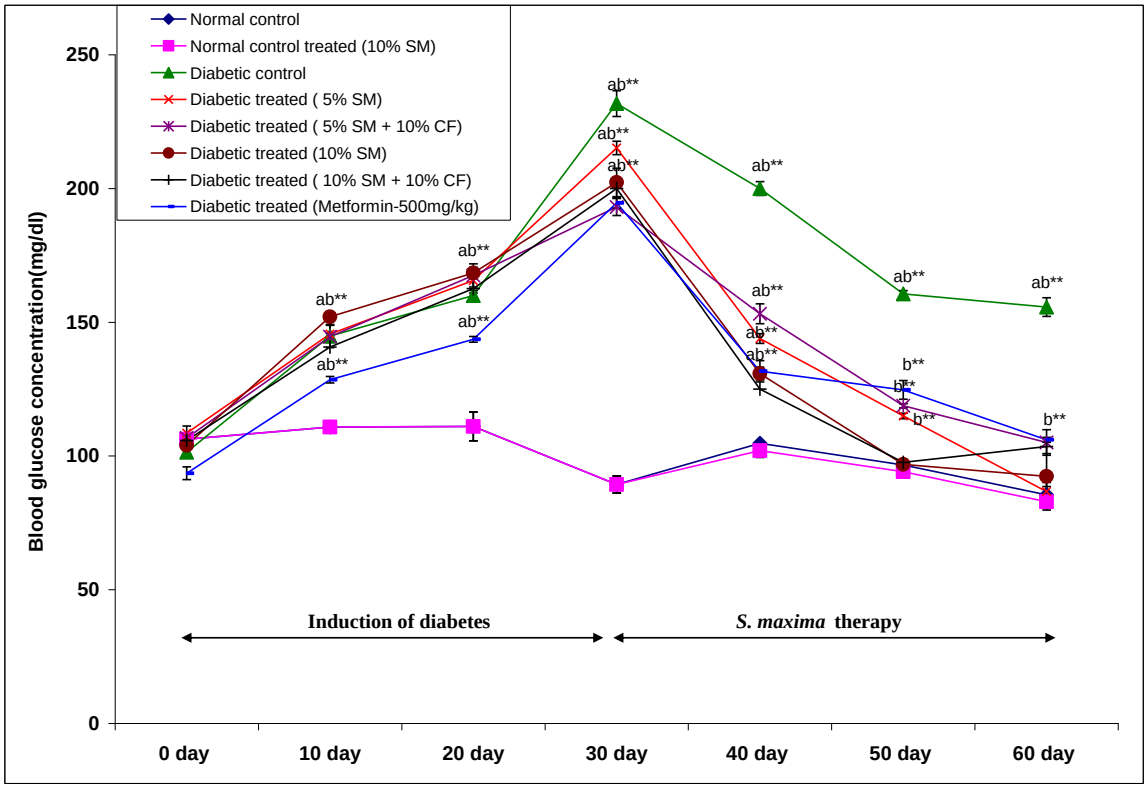


Figure 1: Effect of *S. maxima* extract on blood glucose level in fructose induced hyperglycemia.

Units: mg/dl.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

Effect on hyperlipidemia

The effects of fructose administration and subsequent treatment on triglyceride, cholesterol, HDL-C, LDL and VLDL levels with *S. maxima* are shown in Figs.2 to 4. The following sections discussed these levels individually.

Triglyceride level

The variation of triglyceride level with number of days is plotted in Fig. 4.22. This graph shows the triglyceride level along with the fructose administration and *S. maxima* and metformin therapies. It is observed that the triglyceride level is maximum at 30th day when fructose administration was stopped in four groups while it was continued in two

groups for another 30 days (i.e. upto 60 days), except in case normal control group in which no change was observed. A significant reduction in triglyceride level is obtained on initiation of *S. maxima* therapy and this continued to decline till the period of study i.e. 60 days. An increase of 47.05% was recorded on the 10^{th} day of fructose administration. At the end of 30 days, the triglyceride level were significantly increased by 88.43% ($P<0.001$) (Fig 2.1).The effect of oral administration of *S. maxima* on triglyceride level was studied at two concentrations viz., 5% and 10%. The animals fed with *S. maxima* extract from 30^{th} day onwards showed in all the groups studied herein.

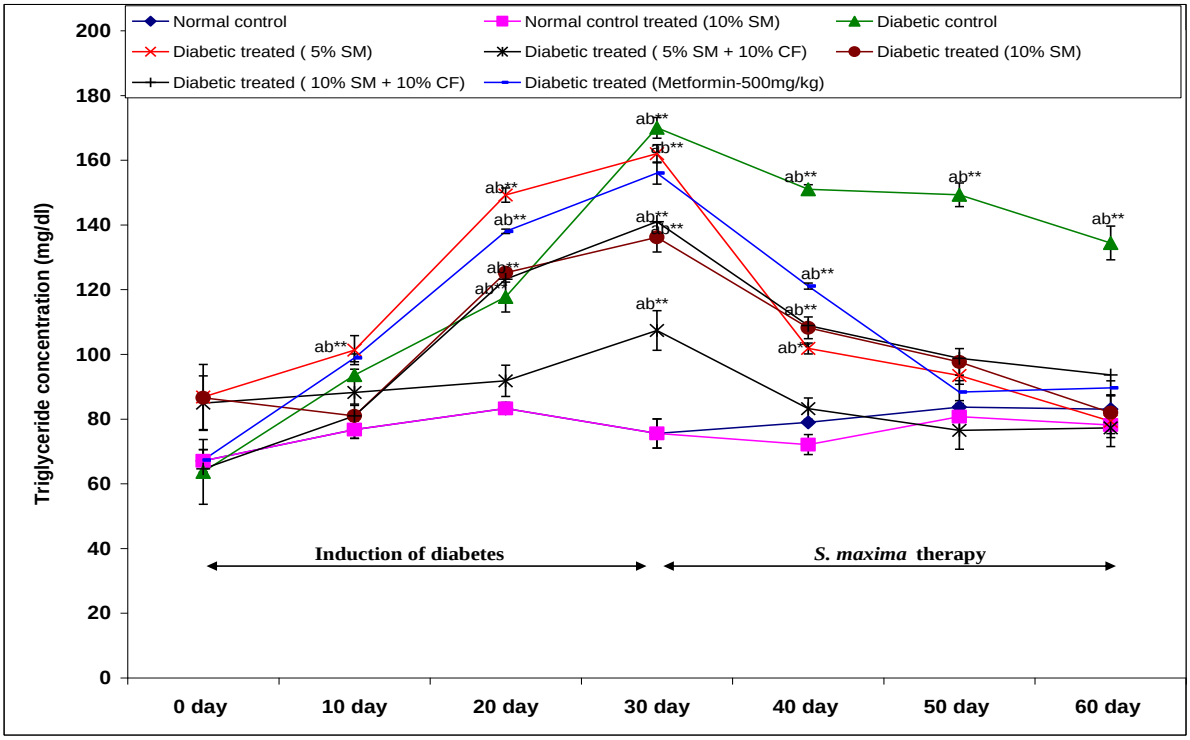
5% S. maxima extract

A significant decrease in plasma triglyceride level was recorded following *S. maxima* therapy. At a dose of 5% *S. maxima*, the triglyceride level was reduced by 51% at the end of 30 days therapy in the group in which fructose was stopped at 30 days, while in continuous fructose fed group, it was reduced by 28.03%. The effect of 5% *S. maxima* extract on normal group was not studied.

10% S. maxima extract

The normal control group fed with 10% *S. maxima* recorded a marginal decrease (3.44%) in plasma triglyceride level. However, the experimental group showed a significant reduction (39%, $P<0.001$) within the 30 days of therapy. In continuous fructose fed rats, *S. maxima* reduced the triglyceride level by 34% at the end of the 30 days therapy. In metformin (500mg/kg) treated group, the triglyceride level was reduced by 42.52% within 30 days of the therapy.

Figure 2.1: Effect of *S. maxima* extract on triglyceride level in fructose induced hypertriglyceredemia.



Units: mg/dl.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

2.2 Cholesterol level

The variation of cholesterol level with number of days is plotted in Fig. 4.23. This graph shows the cholesterol level along with the fructose administration and *S. maxima* and metformin therapies. It is observed that the cholesterol level is maximum at 30th day when fructose administration was stopped in four groups while it was continued in two groups for another 30 days (i.e. upto 60 days), except in case normal control group

in which it decline. The reduction in cholesterol level is obtained on initiation of *S. maxima* therapy and this continued to decline till the period of study i.e. 60 days. The cholesterol level was increased by about 24% following 10% fructose administration in drinking water within 10 days. At the end of 30 days fructose administration, the increase in total cholesterol level was 60.78%. The effect of oral administration of *S. maxima* on cholesterol level was studied at two concentrations viz., 5% and 10%. The animals fed with *S. maxima* extract from 30^{th} day onwards showed decline in all the groups studied herein.

5% S. maxima extract

The cholesterol level was reduced by 33% following 5% *S. maxima* therapy in the group in which fructose was stopped at 30 days as well as in continuous fructose fed group. The effect of 5% *S. maxima* extract on normal group was not studied.

10% S. maxima extract

A significant reduction (33-36%) in blood cholesterol level was observed in both normal and experimental groups following 10% *S. maxima* administration. In continuous fructose fed rats, *S. maxima* recorded a marginal decrease (5%) in cholesterol level. In metformin (500mg/kg) Fig (2.2) treated group, cholesterol level was reduced by 37%. Both *S. maxima* and metformin had similar effect on the cholesterol level.

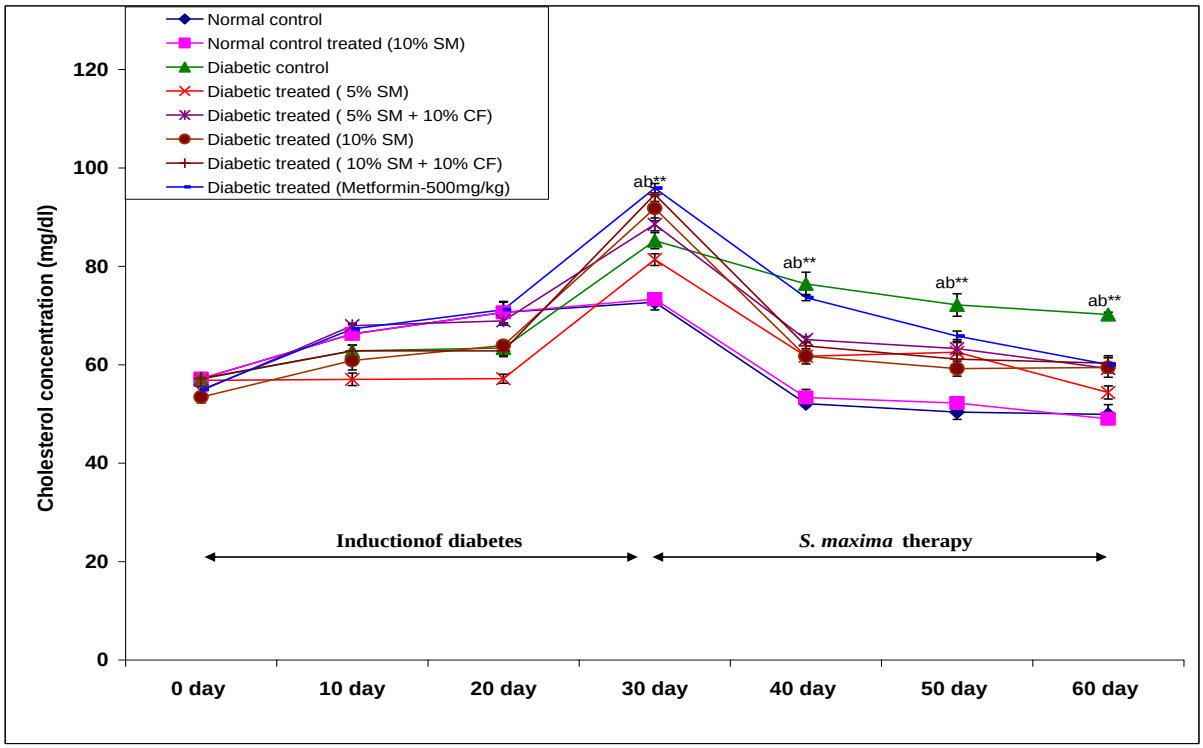


Figure 2.2: Effect of *S. maxima* extract on cholesterol level in fructose induced hypercholesteremia.

Units: mg/dl.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

2.3 HDL-C, LDL and VLDL levels

The variation of HDL-C, LDL and VLDL levels are plotted in Fig. 4.24. This graph shows the HDL-C, LDL and VLDL levels along with the fructose administration and *S. maxima* and metformin therapies. The HDL-C level was reduced by 41.46% while VLDL level was increased by 61.83% in fructose fed animals. The LDL level was increased above 100% after the oral feeding of 10% fructose solution for 30 days to experimental animals. The effect of oral administration of *S. maxima* on HDL-C, LDL

and VLDL levels was studied at two concentrations viz., 5% and 10%. The animals fed with *S. maxima* extract from 30th day onwards showed in all the groups studied herein.

5% *S. maxima* extract

The administration of 5% *S. maxima* extract to the experimental rats (group in which fructose was stopped at 30 days) significantly (P<0.001) reduced the LDL and VLDL levels by 79 and 23% respectively, and elevated the HDL-C level by 55% at the end of 30 days therapy. In the continuous fructose fed group, the LDL and VLDL levels were reduced by 39 and 28% and the HDL-C level was elevated by 43%. The effect of 5% *S. maxima* extract on normal group was not studied.

10% *S. maxima* extract

The administration of 10% *S. maxima* for 30 days to experimental rats (group in which fructose was stopped at 30 days) significantly (P<0.001) reduced the LDL and VLDL levels by 23.91 and 39% respectively, and increased the HDL-C level by 28%. In the continuous fructose fed group, the LDL and VLDL levels were reduced by 30 and 15% and the HDL-C level was increased by 28% within 30 days of therapy. In metformin (500mg/kg) treated group, the LDL and VLDL levels were reduced by 80.10 and 49.18% respectively, while HDL-C level was increased by 53.76%.

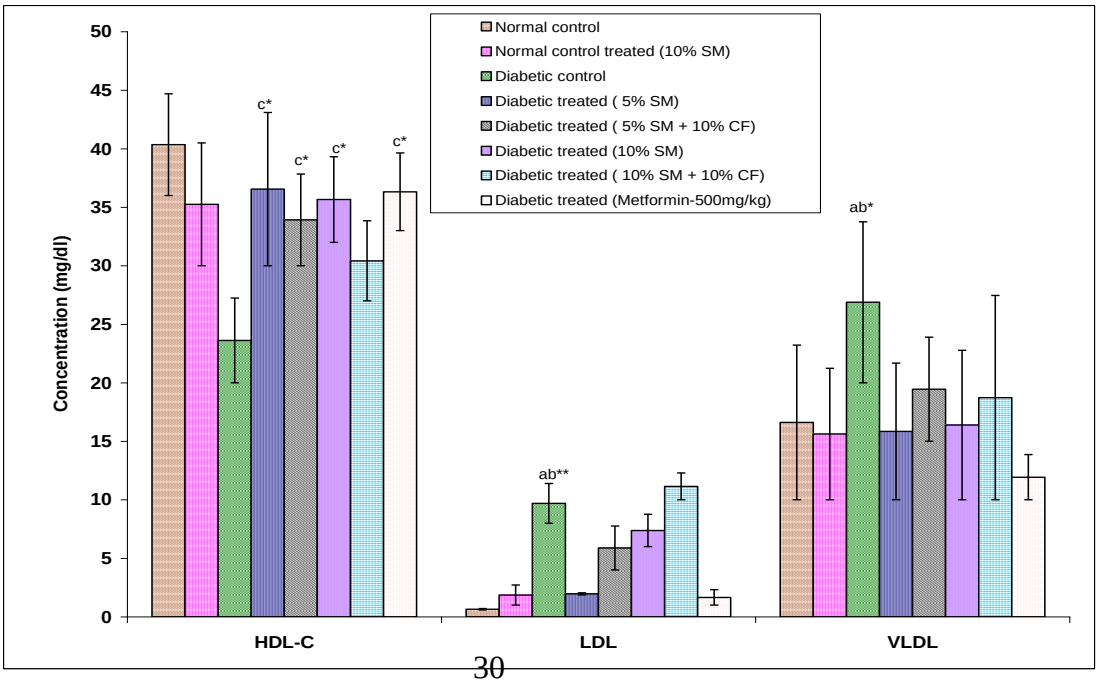


Figure 2.3: Effect of *S. maxima* extract on HDL-C, LDL and VLDL levels.

Units: mg/dl.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

3Effect on liver function markers (SGPT and SGOT)

The variation of liver function markers viz., Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) are plotted in Fig. 3 (a and b). This graph shows the SGPT and SGOT activity along with the fructose administration and *S. maxima* and metformin therapies. The SGPT and SGOT activities were increased by 50.81% and 36.69% respectively in fructose fed rats as compared to the normal animals. The effect of oral administration of *S. maxima* on liver markers was studied by analyzing SGPT and SGOT activities at two concentrations viz., 5% and 10%. The animals fed with *S. maxima* extract from 30th day onwards showed decline in all the groups studied herein.

5% *S. maxima* extract

The markers of liver function were assessed before and after diabetes induction, and at the end of 30 days of *S. maxima* therapy. The administration of 5% *S. maxima* extract to experimental rats (group in which fructose was stopped at 30 days) for 30 days significantly reduced SGPT and SGOT activities by 24.78 and 33.42% (P<0.001) respectively. The reduction was significant even in continuous fructose fed groups. The effect of 5% *S. maxima* extract on normal group was not studied.

10% *S. maxima* extract

The administration of 10% *S. maxima* extract to normal control rats showed a marginal change by 7% in SGPT activity and 4% in SGOT activity. This administration of 10% *S.*

maxima extract to experimental rats (group in which fructose was stopped at 30 days) for 30 days significantly (P<0.001) reduced SGPT activity by 29% (P<0.001) and SGOT activity by 33.57% (P<0.001) respectively. The continuous fructose fed group showed a reduction in SGPT and SGOT activities by 23.03 and 28.27% respectively. The treatment of experimental rats with metformin (500mg/kg) reduced the SGPT and SGOT activities by 14.44 and 19.60% respectively.

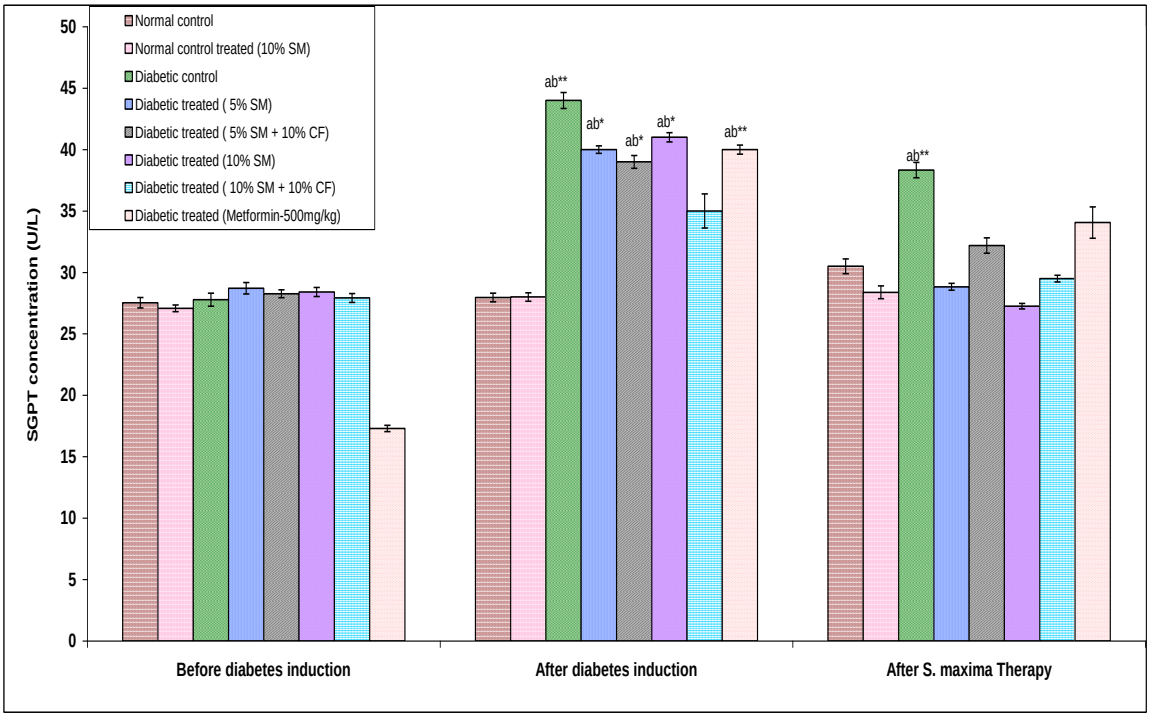


Figure 3(a): Effect of *S. maxima* extract on Serum Glutamate Pyruvate Transaminase (SGPT) activity.

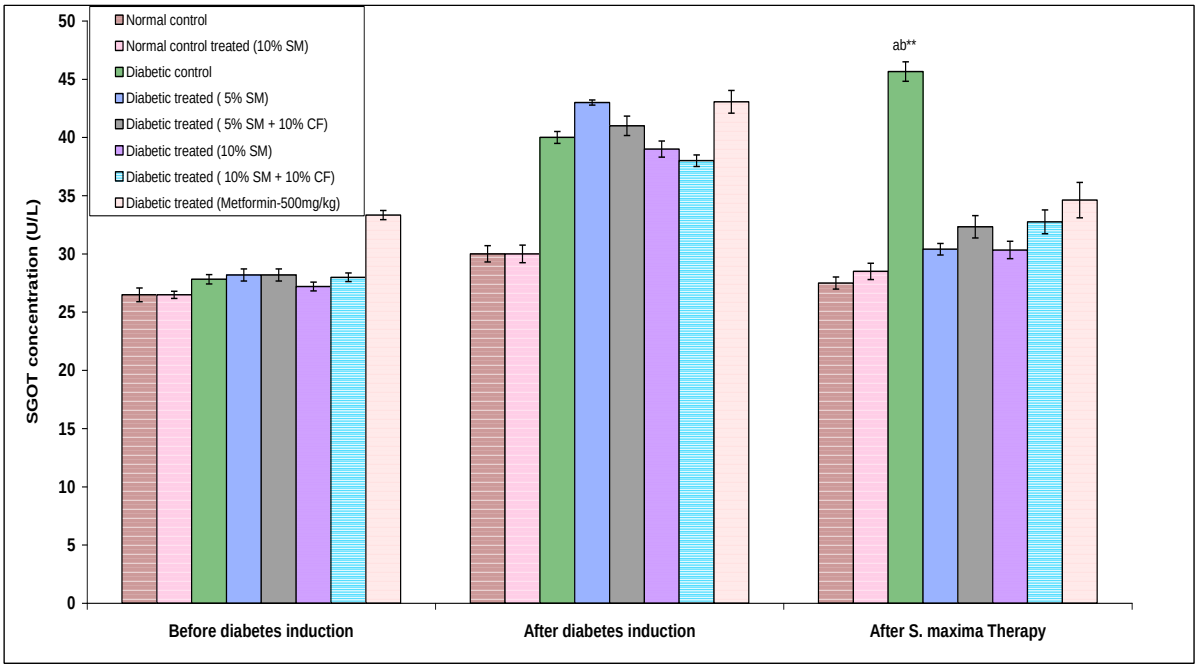


Figure 3 (b): Effect of *S. maxima* extract on Serum Glutamate Oxaloacetate Transaminase (SGOT)activity in fructose-induced hyperglycemia.
Units: unit/L.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

4.Oxidative stress markers in blood

The effects of fructose administration and subsequent treatment on oxidative stress markers [reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and catalase (CAT) with *S. maxima* are shown in Figs. 4.1 to 4.4 The antioxidant activities were significantly affected in all the fructose fed animals. The levels of TBARS, GSH and selected anti-oxidant enzymes SOD and CAT were studied before and after diabetes induction, and at the end of the 30 days *S. maxima* therapy. The following sections discussed these levels individually.

4.1.Reduced glutathione (GSH) level

The variation of GSH level with number of days is plotted in Fig. 4.1. This graph shows the GSH level along with the fructose administration and *S. maxima* and metformin therapy. When the 10% fructose solution was fed for 30 days to Wistar rats, the blood GSH level was reduced by 24.30%. The effect of oral administration of *S. maxima* on GSH level was studied at two concentrations viz. 5% and 10%.

5% *S. maxima* extract

When the experimental rats (group in which fructose was stopped at 30 days) were treated with 5% *S. maxima*, the GSH level was found to be elevated by 40.29% ($P<0.001$) after 30 days of the therapy. In the continuous fructose fed group, the increase was upto 25%. The effect of 5% *S. maxima* extract on normal group was not studied.

10% *S. maxima* extract

A significant elevation in GSH level was recorded in normal (28.50%) as well as in experimental (41.66%) groups. However, the administration of 10% *S. maxima* to continuous fructose fed group increased the GSH level only by 8%. In metformin (500mg/kg) treated group also, the blood GSH level was increased by 8% after 30 days of therapy.

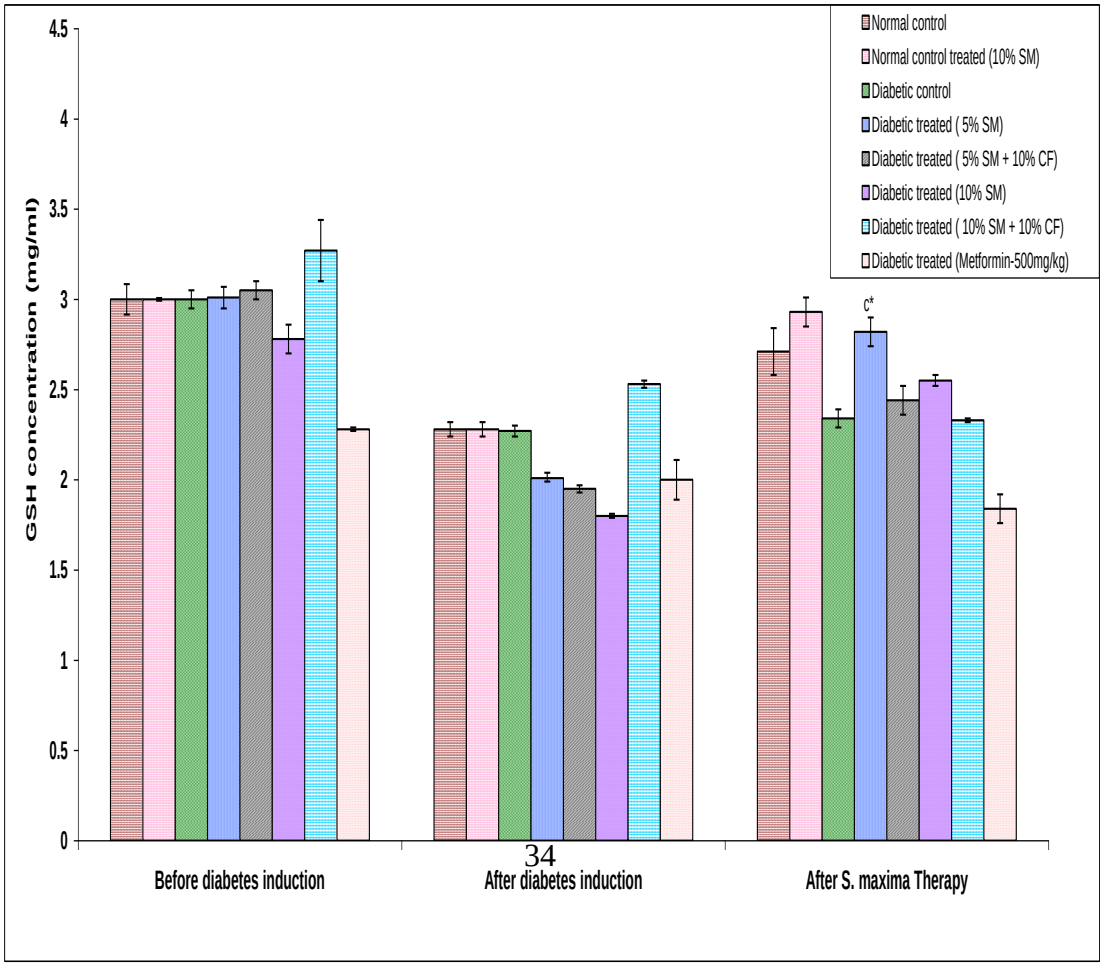


Figure 4.1: Effect of *S. maxima* extract on reduced glutathione (GSH) level.

Units: mg/ml.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

4.2Thiobarbituric acid reactive substances (TBARS) level

The variation of TBARS level is plotted in Fig. 4.2. This graph shows the TBARS level along with the fructose administration and *S. maxima* and metformin therapies. When the 10% fructose solution was fed for 30 days to wistar rats, the blood TBARS level was increased significantly by 35.05% (P<0.05). The effect of oral administration of *S. maxima* on TBARS level was studied at two concentrations viz. 5% and 10%.

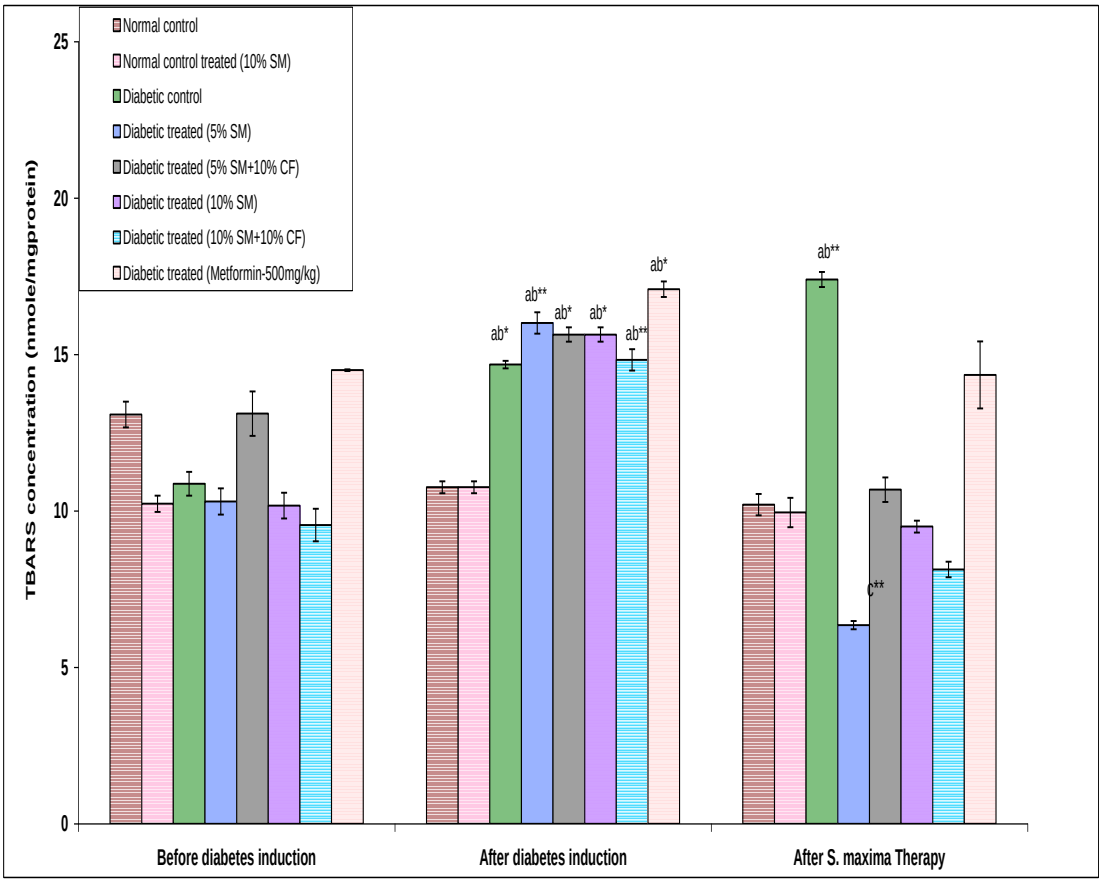
5% *S. maxima* extract

A significant (60.33%) reduction in TBARS level was recorded in experimental group (group in which fructose was stopped at 30 days) after 30 days of 5% *S. maxima* therapy, while, in continuous fructose fed groups it was upto 32%. The effect of 5% *S. maxima* extract on normal group was not studied.

10% *S. maxima* extract

The administration of 10% *S. maxima* significantly reduced the TBARS level inexperimental group (39.25%) while no changes were observed in normal groups after 30 days of the therapy. In the continuous fructose fed groups, the reduction in level was upto 45%. The experimental rats when treated with metformin (500mg/kg) showed reduction in TBARS level by 29.64% after 30 days of the therapy.

Figure 4.2: Effect of *S. maxima* extract on thiobarbituric acid reactive substances (TBARS) level



Units: nmole/mgprotein.

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

4.3 Superoxide dismutase (SOD) activity

The variation in SOD activity is plotted in Fig. 4.3. This graph shows the SOD activity along with the fructose administration and *S. maxima* and metformin therapy. When the 10% fructose solution was fed for 30 days to Wistar rats, the SOD activity was reduced by

55%. The effect of oral administration of *S. maxima* on SOD activity was studied at two concentrations viz. 5% and 10%.

5% *S. maxima* extract

The administration of 5% *S. maxima* to experimental (group in which fructose was stopped at 30 days) and continuous fructose fed groups, increased the SOD activity by 213% ($P<0.001$) after 30 days of the therapy. The effect of 5% *S. maxima* extract on normal group was not studied.

10% *S. maxima* extract

The normal group treated with 10% *S. maxima* extract showed marginal increase of SOD activity (22.32%). The elevation of SOD activity was highly significant in experimental and continuous fructose fed groups (230-320%, $P<0.001$).When the experimental rats were treated with metformin (500mg/kg), there was marginal change in SOD activity (10%).

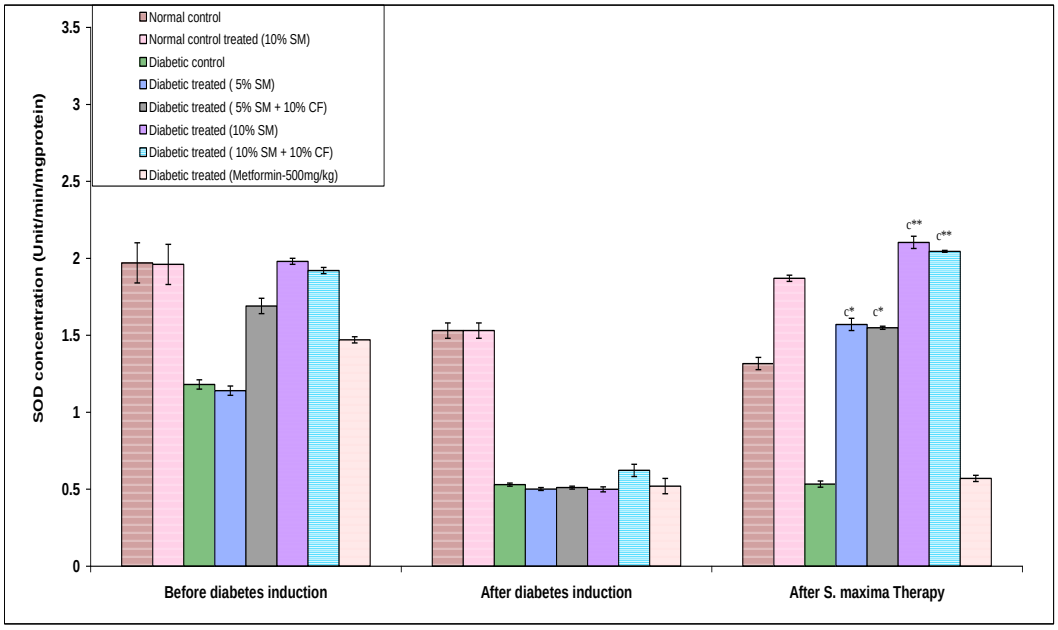


Figure 4.3: Effect of *S. maxima* extract on superoxide dismutase (SOD) activity.

Units: unit/min/mgprotein.

The values were expressed as mean ± SEM of n=5.

[a]$p<0.05$ (*), [a]$p<0.001$ (**) significant compared to normal control group.

[b]$p<0.05$ (*), [b]$p<0.001$(**)significant compared to normal control group along with *S. maxima* administration.

[c] $p<0.05$ (*), $p<0.001$(**) significant compared to DC group.
Values are significantly different by ANOVA Bonferroni t-test.

4.4 Catalase (CAT) activity

The variation in CAT activity is plotted in Fig. 4.4. This graph shows the CAT activity along with the fructose administration and *S. maxima* and metformin therapies. When the 10% fructose solution was fed for 30 days to Wistar rats, the CAT activity was reduced by 23.55%. The effect of oral administration of *S. maxima* on CAT activity was studied at two concentrations viz. 5% and 10%.

5% *S. maxima* extract

The administration of 5% *S. maxima* to experimental rats (group in which fructose was stopped at 30 days) increased the CAT activity by 70.64% ($P<0.001$) after 30 days of the therapy, while in continuous fructose fed groups, the elevation was upto 49%. The effect of 5% *S. maxima* extract on normal group was not studied.

10% *S. maxima* extract

A marginal increase in CAT activity (26.02%) was observed in normal group administered with 10% *S. maxima*. The administration of 10% *S. maxima* to experimental rats increased the CAT activity by 96.29% ($P<0.001$) after 30 days of the therapy, while in continuous fructose fed groups, it elevated the activity by 41%. When the experimental rats were treated with metformin (500mg/kg), the CAT activity was increased by 22%.

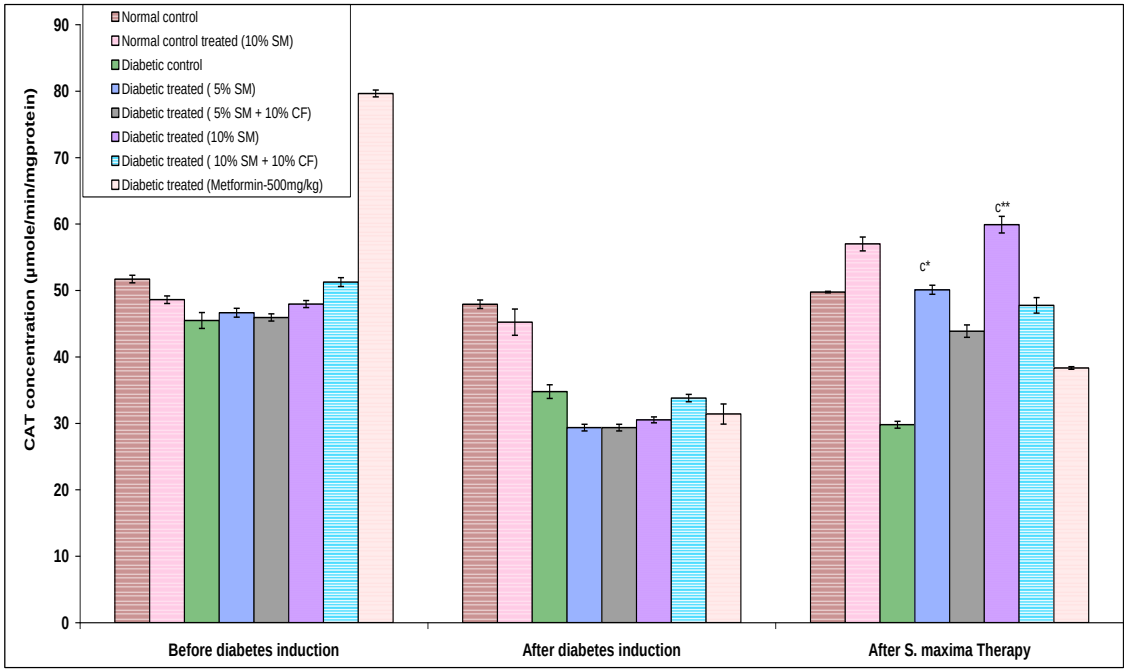


Figure 4.4: Effect of *S. maxima* extract on catalase (CAT) activity.

Units: µmole/min/mgprotein**.**

The values were expressed as mean ± SEM of n=5.

[a]p<0.05 (*), [a]p<0.001 (**) significant compared to normal control group.

[b]p<0.05 (*), [b]p<0.001(**)significant compared to normal control group along with *S. maxima* administration.

[c] p<0.05 (*), p<0.001(**) significant compared to DC group.

Values are significantly different by ANOVA Bonferroni t-test.

Conclusion

The results of the present study show that *S. maxima* brings back the FPG levels (59%) to normal in diabetes-induced rats, i.e. shows antihyperglycaemic activity. It is also evident from the above study that *S. maxima* therapy reduces the fructose induced hypertriglyceridemia by 39%. HDL cholesterol levels increased after one month *Spirulina maxima* therapy by 28%. Significant improvement in liver function markers such as SGOT level, SGPT level were observed after one month Spirulina administration. The Spirulina administration for thirty days enhanced the antioxidant enzymes, catalase (96.29%). The SOD enzyme elevated by 23% after one month Spirulina administration. Spirulina administration reduced 39.25%, the fructose induced elevation of TBARS level.

CHAPTER- 2

EFFECT OF EMF ON THE METABOLITE CONTENT AND THERRAPWUTIC PROPERTY OF S.MAXIMA

INTRODUCTION

Spirulina plankton is a blue green alga found in the highly alkaline lakes of Africa and Mexico, and has been used as food additive because of its high content of proteins as well as essential nutrients like carotenoids vitamins and minerals. *Spirulina* has been promoted as 'the food of the future' with 'exceptional constituents' that contribute to high energy levels. It forms massive population in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate and alkaline pH (up to 11) (Busson., 1971; Clement., 1971). The two most important species of *Spirulina* are *Spirulina maxima and Spirulina platensis. S. platensis* occurs in Africa, Asia and South America, whereas *S. maxima* is confined to Central America and Mexico. It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries (Becker *et al*., 1994). *Spirulina* is cultivated around the world, and is used as a human dietary supplement, available as tablet, flake, and powder form.

The helical shape is maintained only in liquid medium, solid medium filament becomes true spiral (Cifferi., 1983; Tomaselli., 1997). *Spirulina* has a soft cell wall made of complex sugars and protein. The cell wall of *Spirulina* is composed of four layers L-1 to L-4 (Van Eklenburg., 1977). The total wall thickness is about 60 nm and all layers are 10-15 nm thick. The septum separating the cell consists of peptidoglycan, which is coherent with peptidoglycan layer in the cell wall (Stainer and Cohen., 1977). The ultra structure and morphology of *S. maxima* is significantly affected by environmental condition, nutrition factor and composition of medium. Temperature influences the cell size and occurrence of different cell organelles of *Spirulina.*

On a dry-weight basis, the cyanobacterium *Spirulina* is composed of 50–70% proteins, 5–10% lipids and 10–20% carbohydrates (Vonshak., 1997) and possesses an elevated content of compounds of high nutritional value such as provitamins, minerals, polyunsaturated fatty acids such as gamma-linolenic acid (Miranda et al., 1998). *Spirulina* has a 62 % amino acid content and is the world's richest natural source of vitamin B12 and contains a whole

spectrum of natural mixed carotene and xanthophylls phytopigments. *Spirulina* also contains phycobilisomes as light-harvesting protein–pigment complexes. Phycobilisomes are mainly (80–85%) composed of brilliantly colored polypeptides named phycobiliproteins.

Spirulina, with its high concentration of functional nutrients, is emerging as an important therapeutic food. Pre-clinical and clinical studies suggest it has certain therapeutic effects (Fox., 1996), such as reduction in blood cholesterol, protection against some cancers, enhancement of the immune system, increase of intestinal lactobacilli, reduction of nephrotoxicity by heavy metals and drugs, radiation protection, reduction of hyper-lipidemia, and obesity (Belay *et al*., 1993). Ayehunie *et al*. (1998) reported that an aqueous extract of *S. platensis* partially inhibited HIV-1 replication in human T-cell lines, peripheral mononuclear cells, and Langerhans cells. Thus it has been experimentally proven, in vivo and in vitro that it is effective to treat certain allergies (Belay *et al*., 1993), anemia (Belay *et al*., 1997), cancer (Peto *et al*., 1998), hepatotoxicity, viral (Ayehunie *et al*., 1998), cardiovascular diseases, hyperglycemia (Takai *et al*., 1991), hyperlipidemia (Iwata *et al*., 1990; Nayaka *et al*., 1988), immunodeficiency and inflammatory processes (Yang *et al*., 1997; Kim *et al*., 1998), among others. Several of these activities are attributed to some of its components including fatty acids, omega-3 or omega-6, ß-carotene, alpha-tocopherol, phycocyanin, phenol compounds and a recently isolated complex Ca-Spirulan (Ca-Sp) (Hayashi *et al*., 1996).

Spirulina was probably the most widely used in outdoor cultivation trials and there has been over four decades of intensive ecological and physiological research and development using large-scale production units. Owing to its extraordinary nutritional and medicinal qualities, today *Spirulina* cultivation is becoming a worldwide phenomenon. *Spirulina* production may be carried out in closed and open systems. The closed system involves laboratory photobioreactors (Materassi *et al*., 1980; Torzillo &Carlozzi., 1996). The system used commonly for commercial *Spirulina* production are typical large-scale open systems. The open systems involves the open raceway ponds. Open ponds are highly vulnerable to contamination by other microorganisms, such as other algal species or bacteria. According to Chaumont (1993), the two principal advantages of open culture systems are a small capital investment and the use of free solar energy.

The open systems, due to its low production cost, easy handling and high production of biomass, are frequently chosen for industrial production. But reliability, scalability, control, low outputs, large energy & labour inputs are the main problems that in some cases, override the benefits. So the loss of water due to evaporation, threat of contamination, pollution, absence of temperature control and long light path length are the major drawbacks of open systems and this led to search for better alternative (Costa *et al*., 2003; Richmond., 1996).

For solving these problems a variety of enclosed photo bioreactors have been proposed or used for microalgae cultivation (Lee., 1986; Tredici *et al*., 1991; Richmond *et al*.,1993; Ogbonna & Tanaka., 1997). Photobioreactors, despite their costs, have several major advantages over open systems. Closed Photobioreactors are characterized by the regulation and control of nearly all the biotechnologically important parameters i.e. maximum exposure to light, narrow light path length coupled with safe mixing system as well as by following fundamental benefit: reduced contamination risk, no CO2 losses, producible cultivation conditions, controllable hydronamics and temperature and flexible design (Pulz., 1992).

A photo bioreactor can operate in "batch mode", which involves restocking the reactor after each harvest, but it is also possible to grow and harvest continuously. Continuous operation requires precise control of all elements to prevent immediate collapse. The grower provides sterilized water, nutrients, air, and carbon dioxide at the correct rates. This allows the reactor to operate for long periods. An advantage is that algae that grows in the "log phase" is generally of higher nutrient content than old "senescent" algae. Maximum productivity occurs when the "exchange rate" (time to exchange one volume of liquid) is equal to the "doubling time" (in mass or volume) of the algae.

The horizontal tubular photo bioreactors in which the circulation of fluids is induced by bubbling air are now the best accepted. Although the horizontal tubular systems have notable advantages relative to conventional facilities (e.g., open ponds), they also have serious limitations such as difficult temperature control, the need for frequent recarbonation because of the tube length, growth inhibition by dissolved oxygen, foaming, fouling, etc. A possible alternative for overcoming the noted constrains may be the airlift photobioreactor

(ALP). Airlift phtobioreactors allow better gas exchange, a more ordered liquid flow, and hence, a more efficient exposure of cells to light (Pulz., 2001). Hence in the present study S. maxima was cultivated in airlift photobioreactor

Spirulina due to its high concentration of functional nutrients, may be used as a therapeutic supplement for the management of various nutritional and metabolic disorders (Henrikson., 1997; Mani *et al*., 2000).There are some studies which reported that *Spirulina* supplementation (2g/day for 21 days) resulted significant reduction in fasting blood sugar concentration and improvement in the lipid profile of subjects with type 2 diabetes (Mani *et al*., 2000). *Spirulina* administration was also found to reduce the cholesterol levels in hyperlipemic subjects (Henrikson., 1997). Administration of *Spirulina* also enhances the antioxidant potential. Hence present study was focused in screening the blood glucose reduction potential of *Spirulina maxima* cultured under airlift photobioreactor. The biomass of *S.maxima* could be improved through the stress and mutations hence the culture of *S.maxima* was exposed to different EMF intensities for enhanced production of biomass and metabolites.

Spirulina is a photosynthetic, filamentous, spiral-shaped, multicellular and blue-green microalga classified as follows.

Classification of Spirulina maxima

Kingdom - Bacteria

Phylum - Cyanobacteria

Class - Cyanophyceae

Order - Oscillatoriales

Family - Oscillatoriaceae

Genus - Arthrospira (*Spirulina*)

Species - *maxima*

Chemical composition:-

Spirulina is a very rich source of nutrition. It is currently popular as a health food in the U.S. and Europe, often taken as a nutritional supplement in the form of powder or tablet. Since 1970, *Spirulina* has been analyzed chemically. It has been shown to be an excellent source of proteins 60-70 % (Ciferri., 1983), vitamins (Richmond., 1992; Becker., 1984; Belay., 1997), lipids 4-7% (Cohen., 1997), minerals (Pyufoulhoux *et al.*, 2001), and carbohydrates 13.6% (Shekharam *et a*l., 1987).

Therapeutic aspects:-

Several studies have outlined the biochemical composition, immuno-stimulatory and therapeutic potential of *Spirulina maxima* (Hirahashi *et al*., 2002). *Spirulina maxima* contains phenolic acid, tocopherols and β-carotene which are known to exhibit anti-oxidant properties. The extract of *Spirulina* contains a phycobilliprotein that is a potent free radical scavenger and it inhibits microsomal peroxidation (Pinero *et al*., 2001). The *Spirulina maxima* exhibit potent anti-viral activity (Lee *et al*., 2001). Ishii *et al*., (1999) studied the influence of *Spirulina* on IgA levels in human saliva and demonstrated that it enhances IgA

production, suggesting a pivotal role of microalga in mucosal immunity. It is also known to exhibit anti-inflammatory and anticancer properties (Reddy *et al*., 2003). Nakaya *et al*., (1988), in the first human study, gave 4.2 g day^{-1} of *Spirulina* to 15 male volunteers and, although there was no significant increase in high-density lipoprotein (HDL) levels, they observed a significant reduction of high-density lipoprotein (LDL) cholesterol after 8 weeks of treatment. A significant decrease in plasma cholesterol was observed in *Spirulina* fed rats (Gonzalez *et al*., 1993; Torees-duran *et al*., 1999). In an elaborate study involving feeding rats with high cholesterol diets with and without *Spirulina* supplementation, Kato *et al.*(1984) found that the elevation of total cholesterol, LDL+VDL cholesterol, and phospholipids in the serum was reduced significantly when the experimental high cholesterol diet was supplemented with 16% *Spirulina*.

According to Takai *et al.*(1991), a water-soluble fraction of *Spirulina* was found effective in lowering the serum glucose level at fasting while the water-insoluble fraction suppressed glucose level at glucose loading (Takai et al., 1991). Similar results were found in other studies Hosoyyamada et al., 1991; De clair et al., 1995). Mani et al., 1998 reported significant decrease in the fasting blood sugar level of patients was observed after 21 days of 2 g/day *Spirulina* supplementation. According to Takai *et al.*(1991), a water-soluble fraction of *Spirulina* was found effective in lowering the serum glucose level at fasting while the water-insoluble fraction suppressed glucose level at glucose loading (Takai et al., 1991). Similar results were found in other studies (Hosoyyamada et al., 1991; De clair et al., 1995). In a human clinical study involving 15 diabetics, a significant decrease in the fasting blood sugar level of patients was observed after 21 days of 2 g/day *Spirulina* supplementation (Mani et al., 1998). Layam *et al.* reported that the possible mechanism by which *Spirulina* brings about its antihyperglycemic action may be through potentiation of the pancreatic secretion of insulin from islet β-cell or due to enhanced transport of blood glucose to the peripheral tissue. This was clearly demonstrated by the increased levels of insulin and C-peptide in diabetic rats treated with S*pirulina*.

Cultivation of Spirulina:-

Spirulina is currently mass produced as a monoculture in outdoor cultivation systems (Venkatraman et al., 1995) wherein the growth medium utilized forms an important input and accounts for a major share of the costs involved in production of *Spirulina* (Vonshak et al., 1988). The first synthetic medium formulated for cultivation of *S.maxima* was Zarrouk's medium (Zarrouks., 1996) which is still used as the standard medium (SM). Subsequently many media were developed using seawater, sewage water, industrial effluents, CFTRI, Rao's and modified medium. However, over the last few years it has been found to have many nutritional and pharmacological properties.

Factors influencing the growth of Spirulina:-

The different factors affecting the cultivation of *Spirulina* includes the light (Trozillo *et al*., 1991) temperature (Vonshak and Tomaselli., 2000), medium components such as carbon (Ogava & Terui., 1970) , Nitrogen (Fainyuch *et al*. 1991) , phosphorus (Fainyuch *et al*. 1991). In the present study the EMF is applied on *Spirulina* and its effect was monitored in case of its biomass and metabolites.

Effect of EMF on living cells:-

Electromagnetic fields are naturally present on the earth. It is known that the exposure to high intensity EMF in human being can produce several adverse effects such thermal injuries, seizures, nervous and muscle cell excitement; more recently, many studies showed that the association between EMF and childhood cancers such as leukemia, lymphoma and brain tumors (Martin *et al.*, 1996) and immune system effects with altered number of natural killer cells(Tuschl *et al*., 2000), cardiac effects (Savitz *et al*., 1999) and efflux of calcium ions from brain tissue (Blackman *et al*., 1988).

Effects of EMF on bacteria:-

It was reported that microorganisms as bacterial growth and development is influenced by GHz frequency intensity fields (Goodman *et al*., 1994) and also *E. coli* exposed to 100 Hz showed maximal growth when compared to 10 KHz and 1 MHz (Nascimento *et al*., 1996).

Some researches about effect of EMF:-

Singh *et al*. found that magnetic fields with a magnetic flux density of 3000 gauss applied from a permanent magnet for 1-6 h inhibited the growth of *Anabaena doliolum (*Singh *et al*., 1994). Kimball *et al*. found that the growth rate of buds in the Burgundy wine yeast decreased by 20-30% under a magnetic field with the extremely low magnetic flux density of 4 gauss (Kimball *et al*., 1938). Genkov *et al*. reported the acceleration of growth of *Trichomonas vaginalis* at 460-1200 gauss (Genkov et al., 1974). Moore reported that the growth of *Bacillus subtilis* increased by 30%at 150 gauss but decreased by 25% at 300 gauss (Moore.,1979). Takashashi *et al*. investigated the effect of magnetic fields on the growth of *Chlorella* using magnetic fields with magnetic flux densities of 60-580 gauss, and have identified facilitation of growth below 400 gauss and inhibition of growth at 580 gauss (Takahashi *et al*., 1985). Yamaoka *et al*. reported that the growth rate of *Dunaliella salina* and its β-carotene content reached maximal values at 100 gauss (Yamaoks *et al*., 1992).

Morio *et al*. investigated the influence of a magnetic field on photosynthesis in, and the growth of *Spirulina platensis*, under magnetic fields with magnetic flux densities varying from 0.5 gauss to 700 gauss.The specific growth rate of *S. platensis* was the highest at 100 gauss, being 1.5-fold that at 0.5 gauss, while the growth was obviously inhibited at 700 gauss (Morio *et al*., 1998).

Koji Tsuchiya *et al*. reported the growth of *Escherichia coli* under homogeneous 5.2~6.1 T and 3.2~6.7 T magnetic fields which were produced by a newly constructed supereconducting magnetic biosystem (SBS). Those high magnetic fields adversely affected on the growth of the bacterium in the early logarithmic growth phase. However, in the stationary phase, the cell number under a high magnetic field was about 2~3 times higher than that of a control, including that the magnitude of the decrease in the cell number was reduced by the high magnetic field. The effect of the inhomogeneous magnetic

field was much stronger than that of the homogeneous one. The high magnetic field was found to affect the cells of the bacterium differently, depending on the growth phase (Koji Tsuchiya *et al*., 1996).

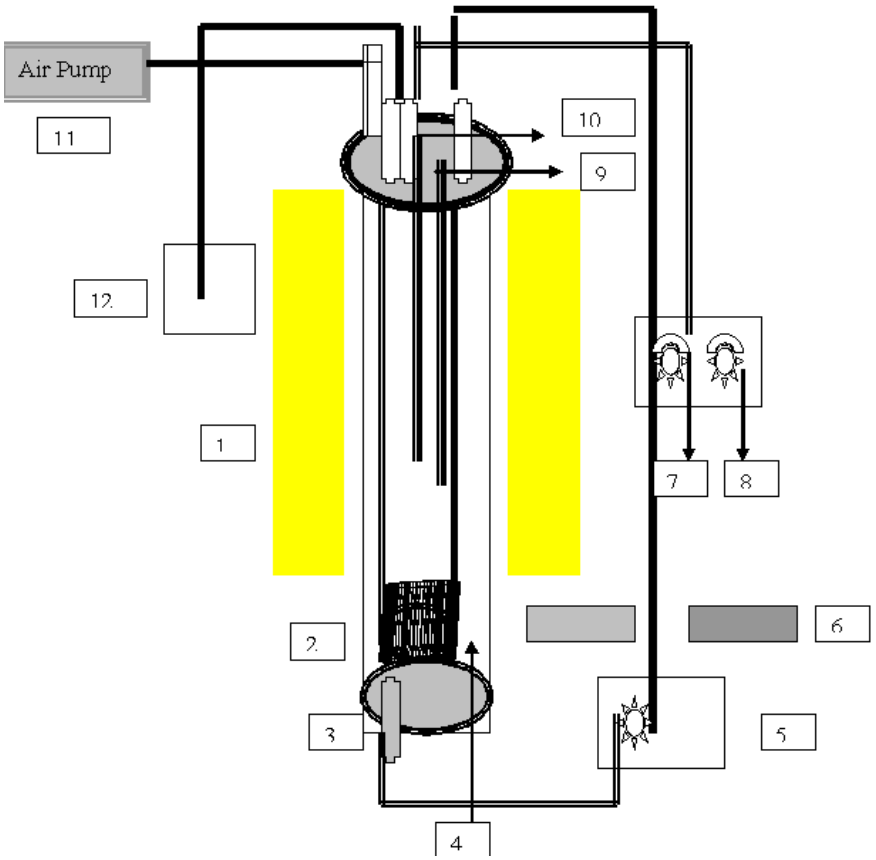
Hence the study is focused on to evaluate the effect of EMF on the growth and metabolites of *S. maxima.*

Spirulina was being administered as a therapeutic agent like antihypercholestrol, antioxidant, antiinflammatory, antidiabetic. It was reported that *Spirulina* has a blood glucose reduction property and this property is attributed to the insulin secreating activity of *Spirulina*. The present work is also focused on evaluating the blood glucose reducing activity of *S.maxima* cultured in airlift photobioreactor exposed to EMF.

Experimental methods

Culturing and maintainance of *Sprirulina maxima*

Spirulina maxima was cultivated in Zarrouks medium (0.5 ml of A_5 Nutrients) in an airlift photobioreactor under the following conditions such as temeperature of 30 $\pm$ 2 C, light intensity 5120 lumens with a photoperiod of 12/12 and a pH of 9.0. The culture was airlifted at a speed of 1 L.P.M. The cells in the motion were exposed to electromagnetic field of different intensities such as 1 G and 400 G**.** The samples were collected at daily (during the light photoperiod).



Schematic design of the Airlift photobioreactor : (1)Light (2)Downcomer (3)Valves for circulation (4) Heating element (5) Peristatic pump (6) Electromagnetic field through Dipole (7) Acid vent (8) Base Vent (controllers) (9) DO sensing (10) pH probes (11) Air circulation tubes

Analytical assays

The cells were harvested and growth pattern in terms of Chlorophyll –a (Chl-a) was studied according to the method of Mackinney (1941). The carotenoid content was calculated according to the method of Caller and Mackinney (1965). The biomass was determined as reported by Kratz and Meyers (1955) using the formula (μ)=2.303 x (log N_2- log N_1/ T_2- T_1) where N_1- Initial chlorophyll content, N_2- Final chlorophyll content, T_1- Initial time and T_2 – Final time. The phycobiliproteins were extracted in phosphate buffer (pH 6.8) and quantified by spectrophotometry (Bennett and Bogorad 1973). The macromolecular

composition of S. maxima such as total protein (Lowry et al. 1951), carbohydrate content (Dubois et al. 1956) were determined

Amino acid analysis:

The amino acid analysis was performed to analyse the changes induced by electromagnetic field . 0.5g of S. maxima was defatted with hexane solvent for 3times.

The resultant residue material was further treated with 0.05 M KCL for 30 min. The supernatant was separated out after centrifugation at 10,000 rpm for 15 min at 4^0C . The sedimented cells were further treated with distilled water for 3 times to remove the debris. The pellet was washed with Nacl four times followed by 0.1 % Na Lauryl sulphate for four times and finally with distilled water for four times. The supernatant was collected and dried. The pellet and the supernatant were subjected to hydrolysis with 6N HCl for 12 hours. Add equal proportion of 5M NaoH and reevaporate it to remove Hcl. The samples were eluted in C18 column 4.6 x 250 mm , 5u column with an isocratic flow of Methanol and water 70: 30 , 0.3 ml/min flowrate. The analytes were analysed using PDA detector of 1024diodes.

Infra red spectroscopy :

The S. maxima was characterized to IR spectroscopic analysis using Fourier transform infrared in shimadzu prestige -21 spectrophotometer. The dried S. maxima exposed or control were ground with KBr poeder and pressed into pellets for FTIR spectra measurement in the frequency range of 100-4000 cm^{-1}.

Therapeutic dose of *S. maxima*

The therapeutic dose of *Spirulina maxima* of *S. maxima* (500mg/kg, protein content) was prepared by sonication under ice cold condition at 2 pulse/ min for 15 min. The disrupted

material was filtered through whatmann No.1 filter paper and protein content was assayed in the filtrate by the method of lowry et al (1951). The doses were prepared according to the protein content of the material.

Rapid *in- vivo* screening of blood glucose elimination

The drug of interest was administered orally before or after the bolus glucose of 2 g /kg B.wt was administered orally and reduction in blood glucose level was observed at hourly intervals. The animals taken for the study was grouped based on their fasting blood glucose levels**.** The same was carried out in diabetic rats.

Fructose induced insulin resistance

The fructose induced insulin resistance is caused when the animals are kept on the long term administration either in the form of feed or in water. The animals are kept on the fructose enriched diet 72 % along with yeast extract and casein. The blood glucose levels were monitored at weekly intervals.

Results:

There are many reports on the influence of magnetic fields with low magnetic flux densities on microorganism and many of them focused on their influence on the growth of microorganisms. The influence of magnetic intensities (460- 1200 G) was also studied on microorganisms such as *Bacillus subtilis, Trichomonas vaginalis*. The influence of Magnetic intensities up to 3000 G, 700 G , 580 G had already been studied on *Anabena doliolum, S. platensis, Chlorella respectively.* The present study was focused on the growth and productivity of *S.maxima* exposed to lower EMF in an airlift photobioreactor. It was observed from the study that *S.maxima* exposed to the EMF showed the maximum celll density. The maximum cell density of 0.983 abs was observed on the 9^{th} day The maximum specific growth rate per day μ 0.655 was observed per day whereas the control showed a specific growth rate of μ 0.655 however the higher intensity of Electromagnetic exposure to the *S. maxima* considerably reduced the specific growth rate.

The phototrophic cells communicate through the source of Electromagnetic fields hence the light harvesting pigments such as chlorophyll-a, and accessory harvesting pigments such as phycocyanins, phycoerythrins and allophycocyanins, carotenoids were monitored day wise. It was observed from the study that Chlorophyll-a content of *S. maxima* was significantly (P=0.001) elevated by 51.46% on 9^{th} day with respect to the control. Whereas 400 G of EMF exposure to *S.maxima* culture showed detrimental effect on the chlorophyll-a content.(Fig 3).

The carotenoid content was found be elevated significantly in 1 G EMF exposed *S. maxima* and a maximum of 7.20 mg/ml was observed on the 9^{th} day (Fig 4). The phycocyanins was found to be high in 1 G EMF exposed *S. maxima* that is 0.234 mg/ml however the levels were non significant (Fig 5). Allophycocyanin levels were found to be elevated 1 G EMF exposed *S. maxima* (0.338 mg/ml) (Fig 6). Whereas the maximum phycoerythrin levels were observed in the control *S. maxima* however the levels were found to be non significant.

Macromolecules of *S. maxima* such as proteins, carbohydrates were found, carbohydrate content was found to be significantly elevated in non emf exposed cultures. Maximum

carbohydrate content was observed on 9th day (46.43µg/ml) (Fig 8). Similarly the total protein content was also found to be significantly increased in non EMf exposed *S. maxima* (Fig 9).

The morphometrical as well as structural organelle analysis was performed to establish the promotive effect of Effect of EMF 1G, the results indicate that EMF exposures may ccause a change in the membrane arrangent pattern of *S. maxima*. The cell wall membranes of Spirulina (L1 – L!V) held compactly and very rarely appear separated from each other, The formation of the septum as an extension of LII layer Fig (10) between the trichomes is another observation. The uniform arrangement of pigments chlorophyll and other accessory pigments responding to EMFs, whereas the non EMF exposed cultures showed a well constricted form of membranes (LI-LIV). Moreover a large number of storage granules were obsereved in non exposed EMF as result of high carbohydrate content. (Fig 11)

The amino acids of *S. maxima* was evaluated to understand the influence of Electromagnetic field on the cell wall aminoacids of *S. maxima*. It was observed that that EMF 400 G exposed *S. maxima* exhibited the amino acid phenyl alanine which was found to be absent in EMF 1 G exposed or control *S. maxima*. Glycine and serine were the amino acids that was found only in the control *S. maxima*. However there were also some occurrence of cell wall carbohydrate monomers along with the amino acid residues that remain uncharacterized.

The intracellular aminoacids fingerprint of *S. maxima* revealed that EMF exposure didn't make any change in the amino acid composition, however the influence of light intensity had some change with the amino acid composition of *S. maxima*. Methionine was found in the 1G with 10000 lumens exposed *S. maxima* and 400 G exposed *S. maxima* at 5000 and 10000 lumens exposed. Phenyl alanine was found only in *S. maxima* exposed to 400 G or control at 5000 lumens or 10000 lumens. Glycine was observed in 400 G exposed *S. maxima*. Serine was observed only at control and 1 G EMF exposed *S. maxima* at 5000 lumens. Glutamic acid and glutamine were found only with EMF exposed S. maxima however Control S. maxima at 10000 lumens showed the presence of glutamic acid and glutamine. However there were also some occurrence of cell wall carbohydrate monomers along with the amino acid residues that remain uncharacterized.

IR results showed that there is a considerable decrease in the molecules in the spectra of 400 G exposed *S. maxima* especially at the spectral region at 3000- 4000 cm^{-1} . It was reported that protein exhibited the peaks at 3300- 3500cm^{-1} which is understood that the amine groups N-H stretch between 3300-3500cm^{-1}. The peaks are exhibited on 3631 in case of the 400 G EMF exposed *S. maxima* whereas 1 G exposed *S. maxima* exhibited peaks 3049,3267,3604.96 cm^{-1} however the control exhibited peaks at 3074,3271,3468,3597 cm^{-1}. It was observed that sulfhydrl esters have the peak rage of 500-540. The similar peak was exhibited at 549 cm^{-1} in the control as well as 400G EMF exposed S. maxima whereas the 1 G EMF exposed *S.maxima* exhibited a peak of 532 cm^{-1}. The carotenoids had a spectractral 980-915, however EMF exposed as well as control showed a spectral range of 1053-993. It was known from the literatures that Eseters , carboxylic acids have its stretch from the waveband of 1260-1000m^{-1}, EMF exposed or control *S. maxima* exhibited, the spectral bands of 1271-1240 cm^{-1}. The EMF exposed *S.maxima* exhibited 1240 cm^{-1}, which indicates the presence of sulfated polysachharides.

The FTIR spectra of the pure lipid compounds are more complex compared with the respective NIR spectra. Three distinct absorption bands are apparent, of which the CH3 and CH2 (3,025–2,954 cm−1) and the C=O ester (1,746–1,654 cm−1) are most characteristic for lipids. Furthermore, the hydroxyl and phosphate. groups from for example the phospholipids can be distinguished (1,200–500 cm−1). From these individual lipid spectra, it is clear that characteristic and distinct fingerprints for triglycerides and phospholipids exist in the FTIR spectrum

Functional Evaluation of *S. maxima*

Diabetes mellitus comprises a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both; long term

it is associated with permanent damage, dysfunction, and failure of various organs. Several pathogenic processes are involved in the development of diabetes, ranging from autoimmune destruction of pancreatic β-cells (type 1) with consequent insulin deficiency to abnormalities that result in insulin resistance. Type 2 diabetes, which accounts for 90% to 95% of those with diabetes, is among the most common of chronic diseases and comprises individuals who have insulin resistance. The present study is focused in evaluating the blood glucose lowering potential of *Spirulina maxima* in wistar rats fed with high fructose diet. The animals were fed with the fructose rich diet for 6 months. Their fasting blood glucose levels were elevated up to 32.78 % (Fig-12).The protein content of *S. maxima* was quantified and 500mg protein equivalent of *S. maxima* was used as therapeutic dose. The same group of animals were administered with EMF exposed *S. maxima*. It was observed that EMF unexposed cultures (EMF control) of

S. maxima showed a high percentage reduction in the blood glucose levels.

The entire experimental set up was divided into two phases, phase: 1 effect of *S. maxima protein* extract on the blood glucose lowering activity in overnight fasted animals. In this study maximum blood glucose lowering effect was observed with EMF unexposed group in hyperglycemic rats. Hyperglycemic rats showed a significant reduction ($P<0.05$) by 16.57% after 1hour administration of EMF unexposed *S. maxima* and the reduction in blood glucose continued till 3 hour(37.14%) . Whereas similar type of reduction was also observed with diabetic control group. It was observed from the study that, administration of EMF exposed (400 G) *S. maxima* hardly showed blood glucose reduction (fig-10). The blood glucose levels monitored during (0-180 min) revealed the highest AUC (2250 mg/ml.min) with diabetic control group whereas EMF unexposed *S. maxima* administered group showed AUC of 1260 mg/ml.min (fig-16).

The control studies made on the normal animals O/N fasted revealed that normal rats administrated with EMF unexposed *S. maxima* showed a reduction in blood glucose level only after 2 hour (7.22%) which is not significant at $P<0.05$. Normal control group animals showed a continuously reduction in blood glucose level and maximum reduction was observed at 3 hour (24.72%) similar type of glycemic pattern was observed when administered with *S. maxima* exposed to EMF (Fig-1). Highest area under curve was

observed with EMF unexposed *S. maxima* (740mg/dl.min) whereas the AUC of EMF exposed (400 G) *S. maxima* administered group was 684 mg/dl.min (Fig-14).

Phase: 2 effect of *S. maxima* protein extract on the blood glucose lowering activity in non-fasted animals. The blood glucose levels were found to decrease immediately after the administration of *S. maxima* (Fig-16). It was observed from the study, maximum blood glucose reduction was observed with EMF exposed (400 G) by 36.2 % ($P<0.05$) whereas the reduction level was found to be significant with that of control (fig-17) . The total area under the curve shows the total reduction in the blood glucose reduction every hour. It was observed that EMF unexposed S. *maxima* showed the highest area under curve of 1560mg/dl.min whereas the AUC of EMF exposed 400 G was 1500 mg/dl.min (fig-20). The diabetic control animals showed a decrease in blood glucose level at hourly interval but the reduction was not significant.

It was observed from the control studies made on the normal animals that EMF unexposed as well as EMF exposed (400 G) *S. maxima* reduced the blood glucose levels within 1 hr by 14.29 % and 22.36% respectively . The blood glucose levels were hardly found to reduce with normal control group. The results were in accordance with total area under curve (blood glucose curves) being plotted for every group every hour (fig-18).The study concludes that low intensity of electromagnetic field have a role in enhancing the biomass and growth rate of *S. maxima*. Diabetic animals treated with EMF unexposed *S. maxima* exhibited the maximum reduction in the blood glucose level however the reduction was found to be insignificant with that of EMF exposed (400 G), whereas in normal subjects administration of EMF exposed (400 G) exhibited the maximum blood glucose reduction.

Mechanism of Blood glucose Reduction

Insulin levels monitored in nonfasted diabetic animal revealed that, the blood glucose homeostasis was maintained after 2 hrs and the levels of the Insulin was also elevated in rats administered with EMF exposed (5.56 ng/ml) and 3.54 ng/ml in control *S.maxima* administered.

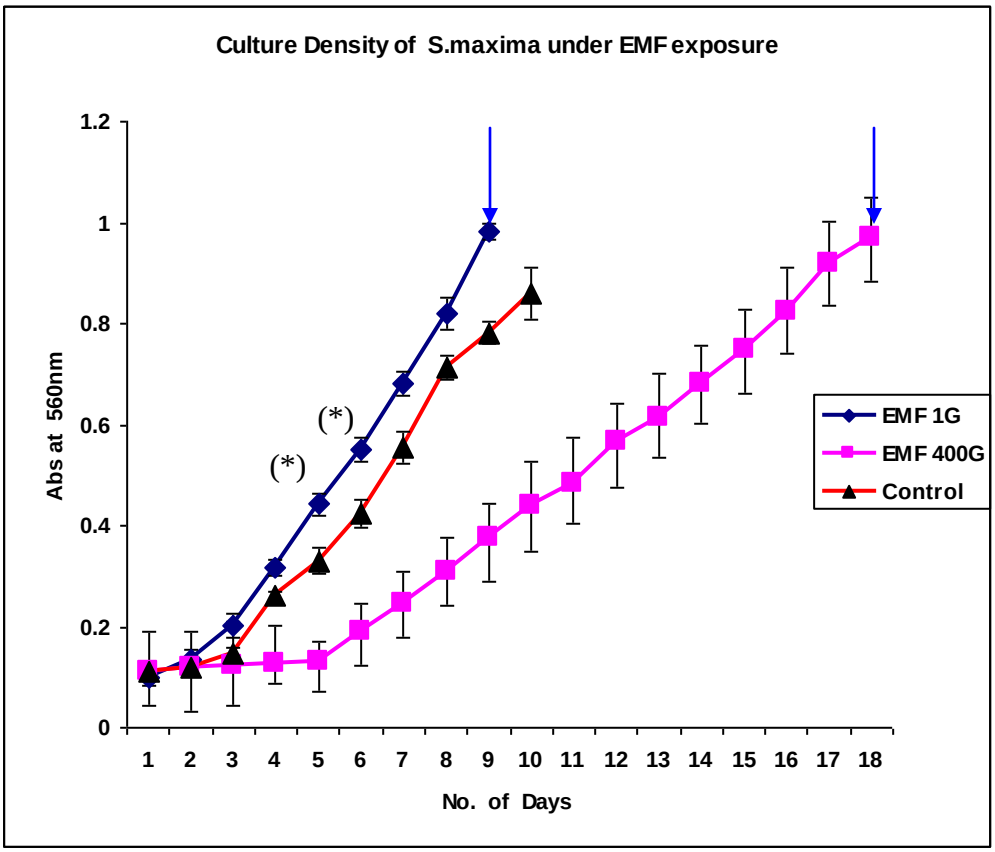


FIG- 6.1: CULTURE DENSITY OF *S. MAXIMA* IN AIRLIFT PHOTOBIOREACTOR UNDER ELECTROMAGNETIC EXPOSURES
The Y- axis denotes the culture density, Unit expressed in nm
X- axis denotes the number of days.
The Unit expressed as mean±S.E

The Down ward arrow indicates the day of harvest when the culture reaches its maximum absorbance.

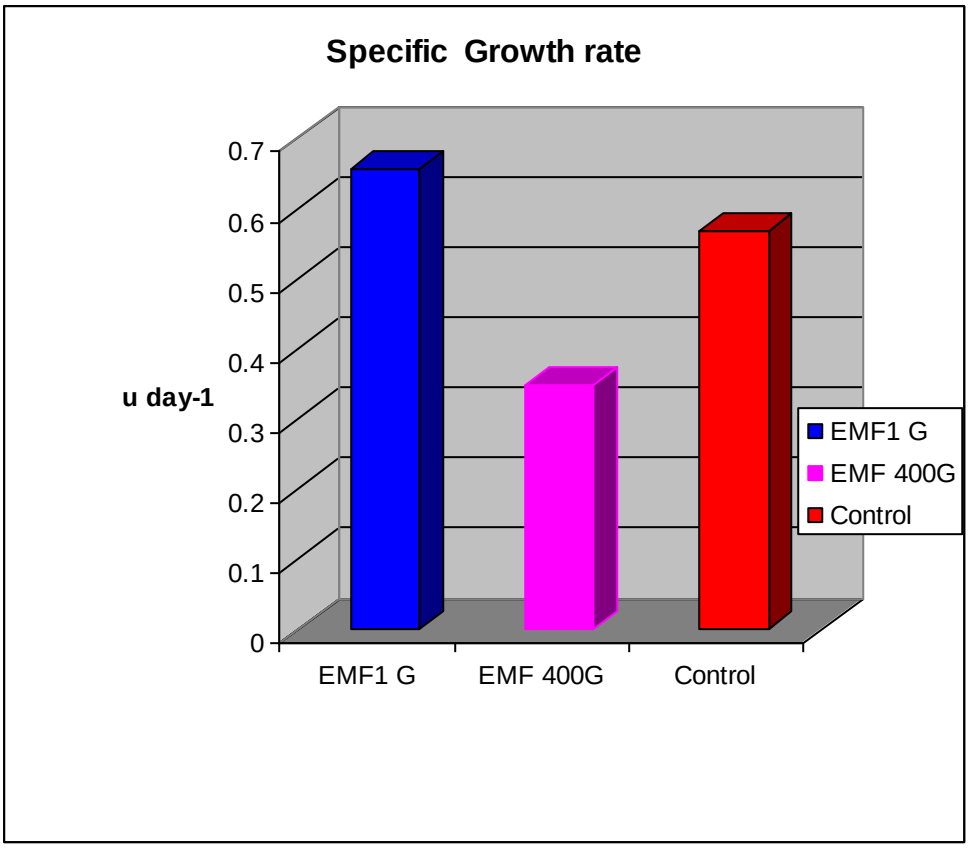


Fig -6.2: THE SPECIFIC GROWTH RATE OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER ELECTROMAGNETIC EXPOSURES

The specific growth rate calculated using the Kratz and Meyers formula and the unit expressed as μday^{-1}

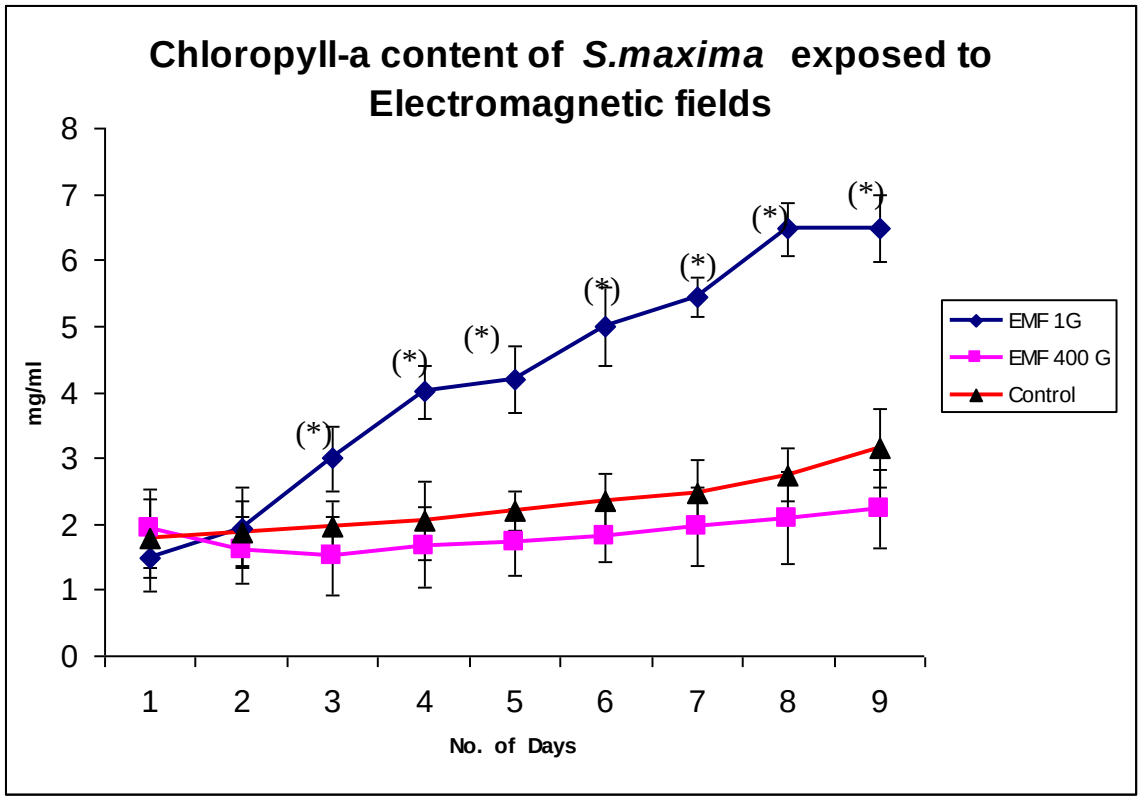


FIG -6.3: CHLOROPHYLL- A CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The chlorophyll unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups.

Statistical significance was observed in the levels of chlorophyll-a from the 3rd day onwards in EMF exposed *S. maxima*

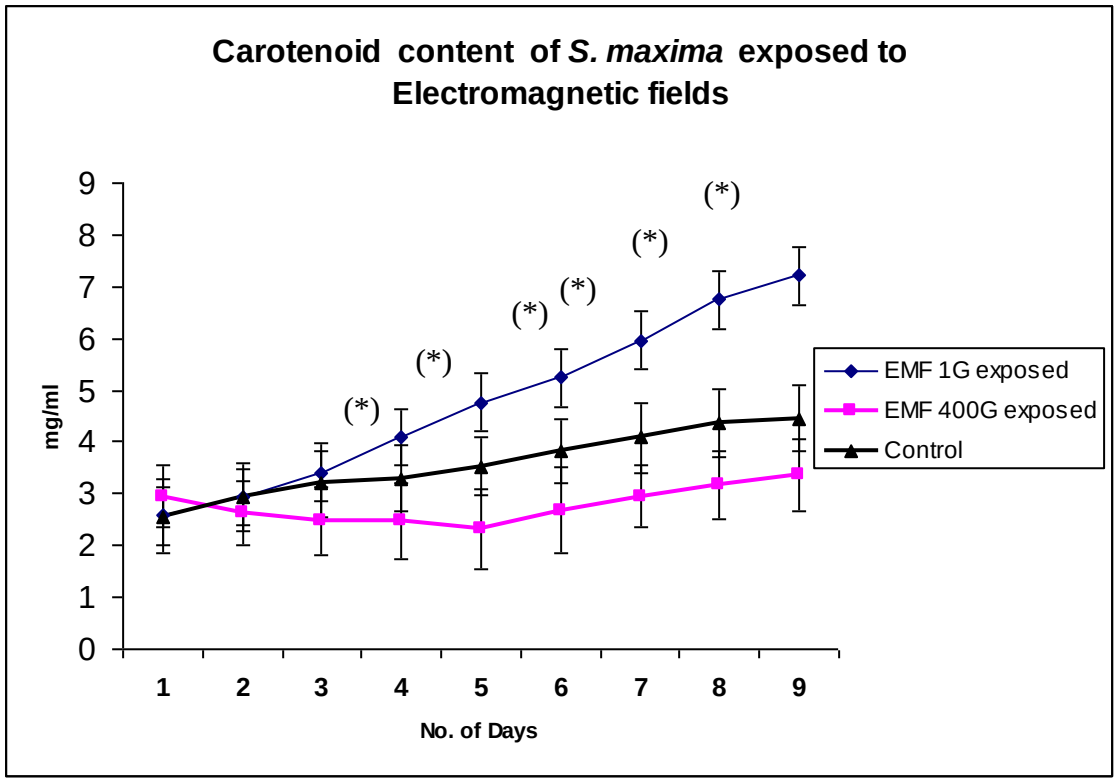


Fig-6.4: CAROTENOID CONTENTOF S. *maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The carotenoid content unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups.

Statistical significance was observed in the levels of carotenoids from the 3rd day onwards in EMF exposed *S. maxima*

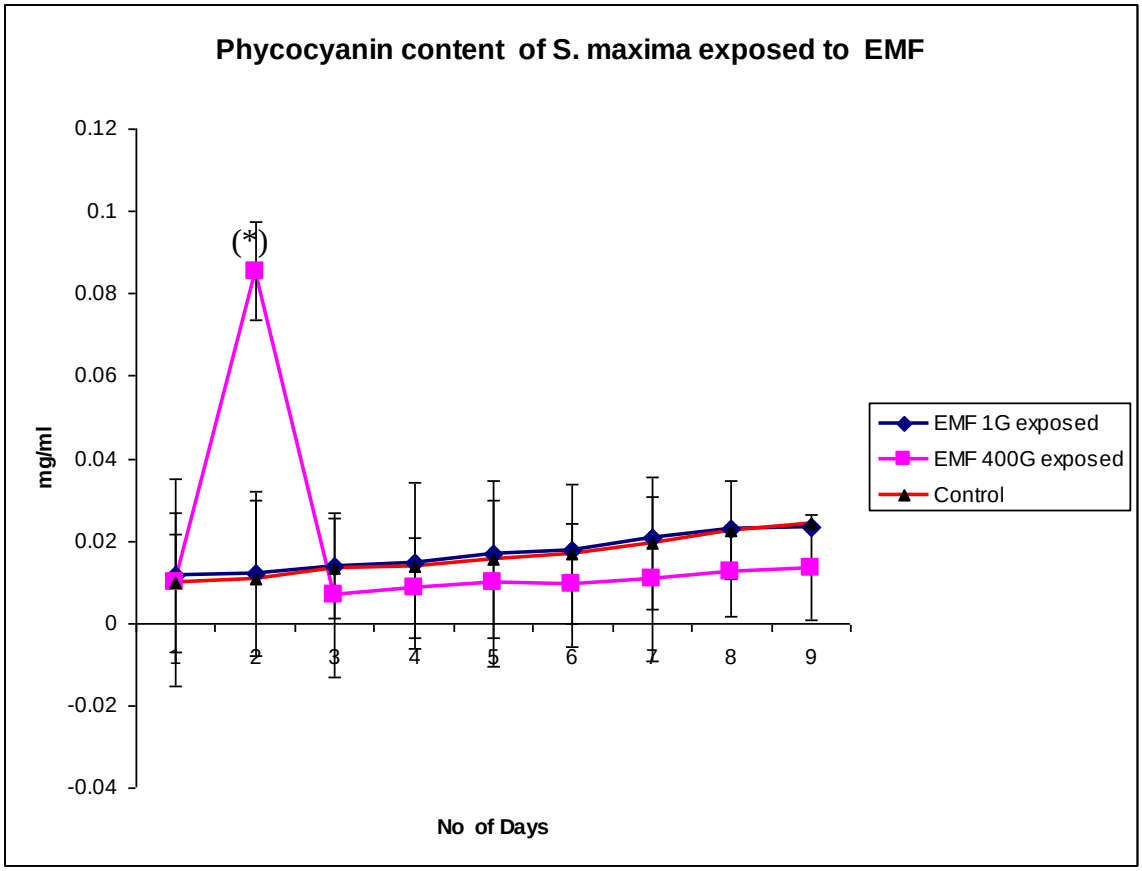
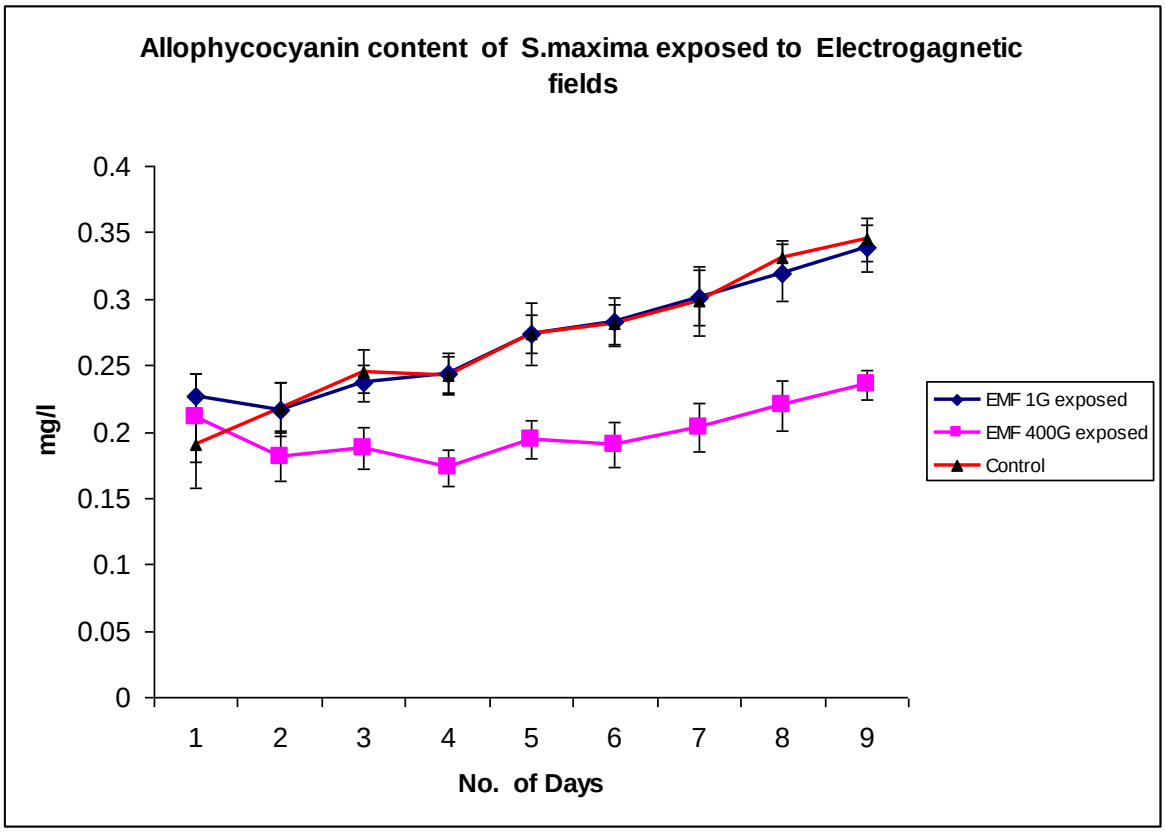


Fig-6.5: PHYCOCYANIN CONTENTOF S. *maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The phycocyanin content unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups.



Fig;6.6: ALLOPHYCOCYANIN CONTENTOF S. *maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The allophycocyanin content unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

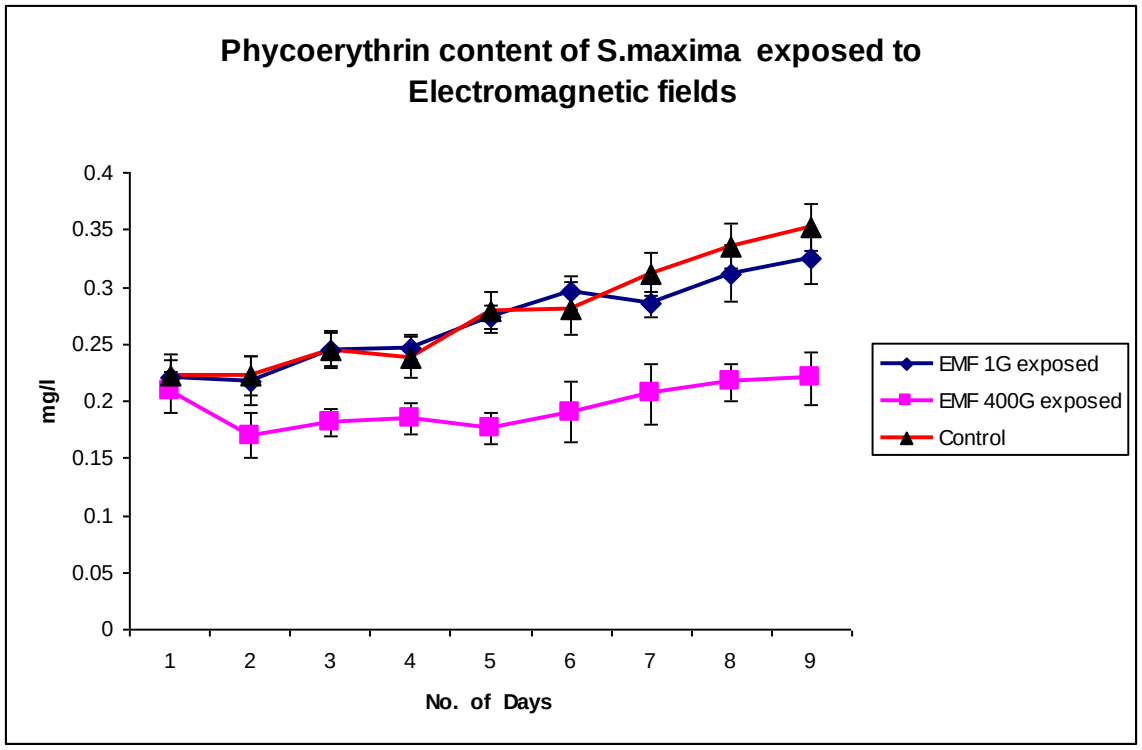


Fig-6.7 PHYCOERYTHRIN CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The phycoerythrin content unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

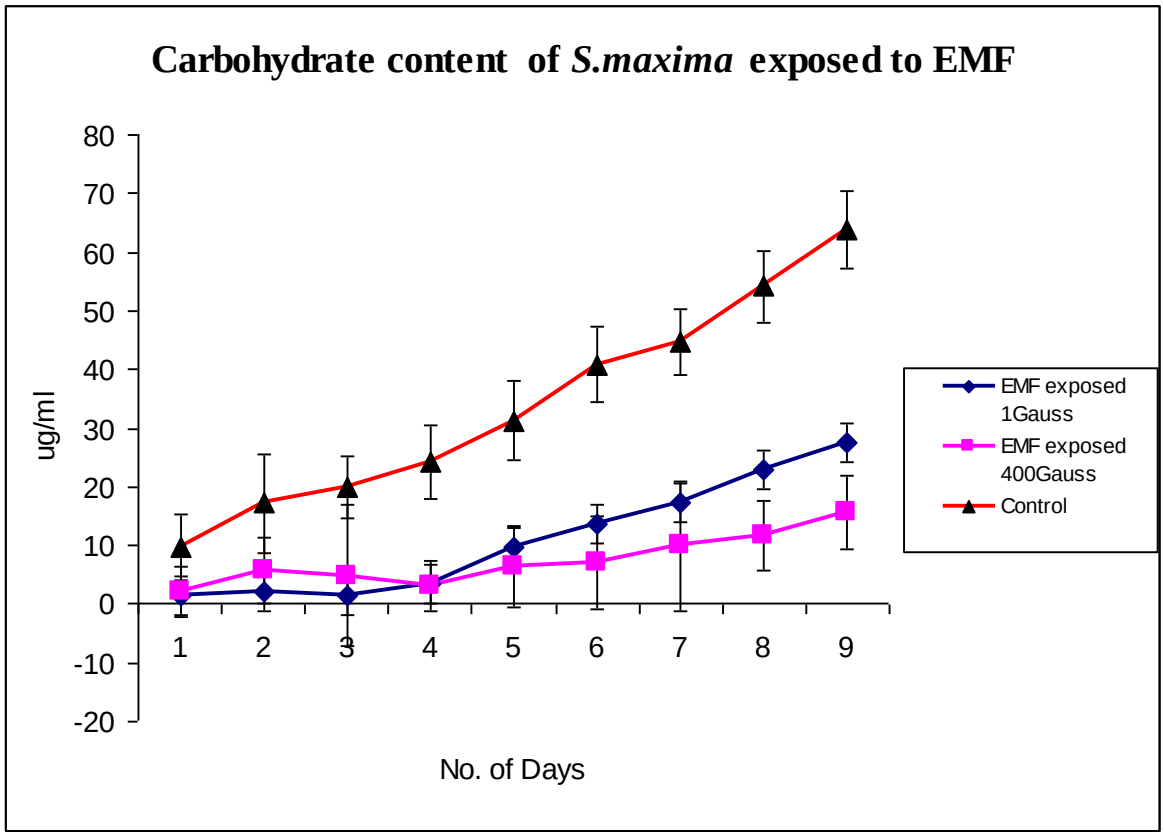


Fig-6.8: TOTAL CARBOHYDRATE CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The total carbohydrate content unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

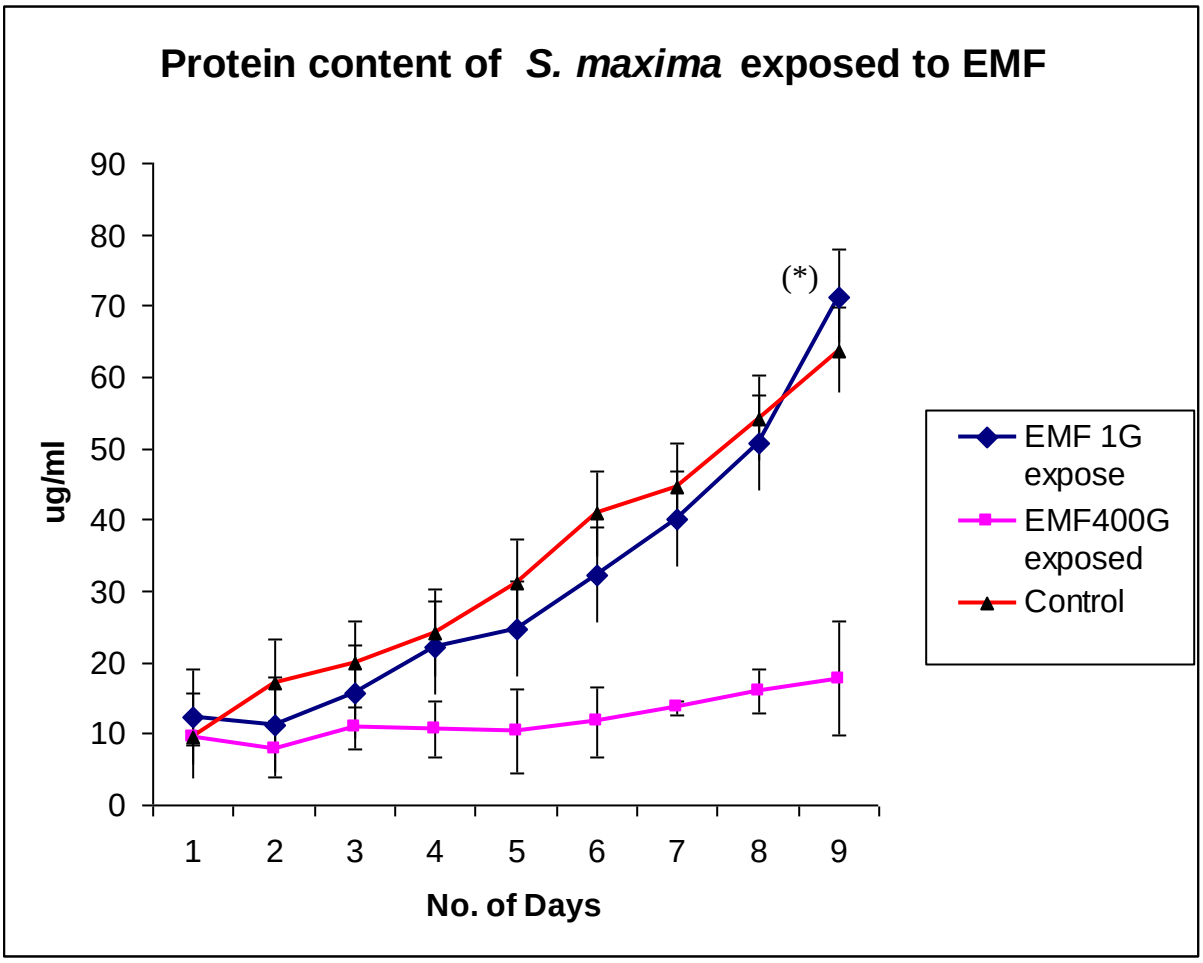


Fig-6.9: TOTAL PROTEIN CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE.

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

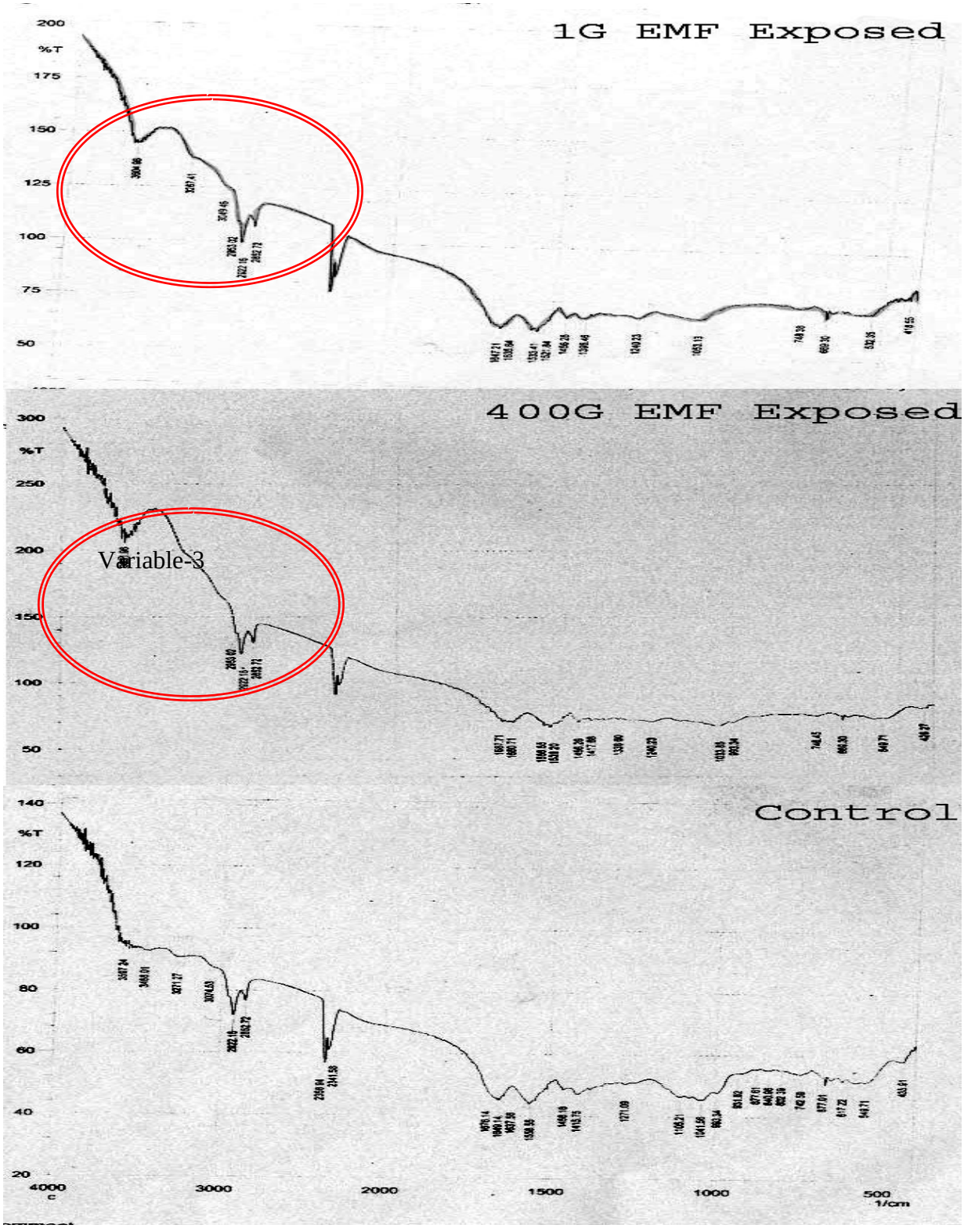

1G EMF Exposed
400G EMF Exposed
Variable-3
Control
%T
1/cm

FIG -7.0: FTIR FINGERPRINT OF *S.MAXIMA* EXPOSED TO EMF

X-axis Unit expressed as cm^{-1}
Y- axis Unit expressed as %Transmittance

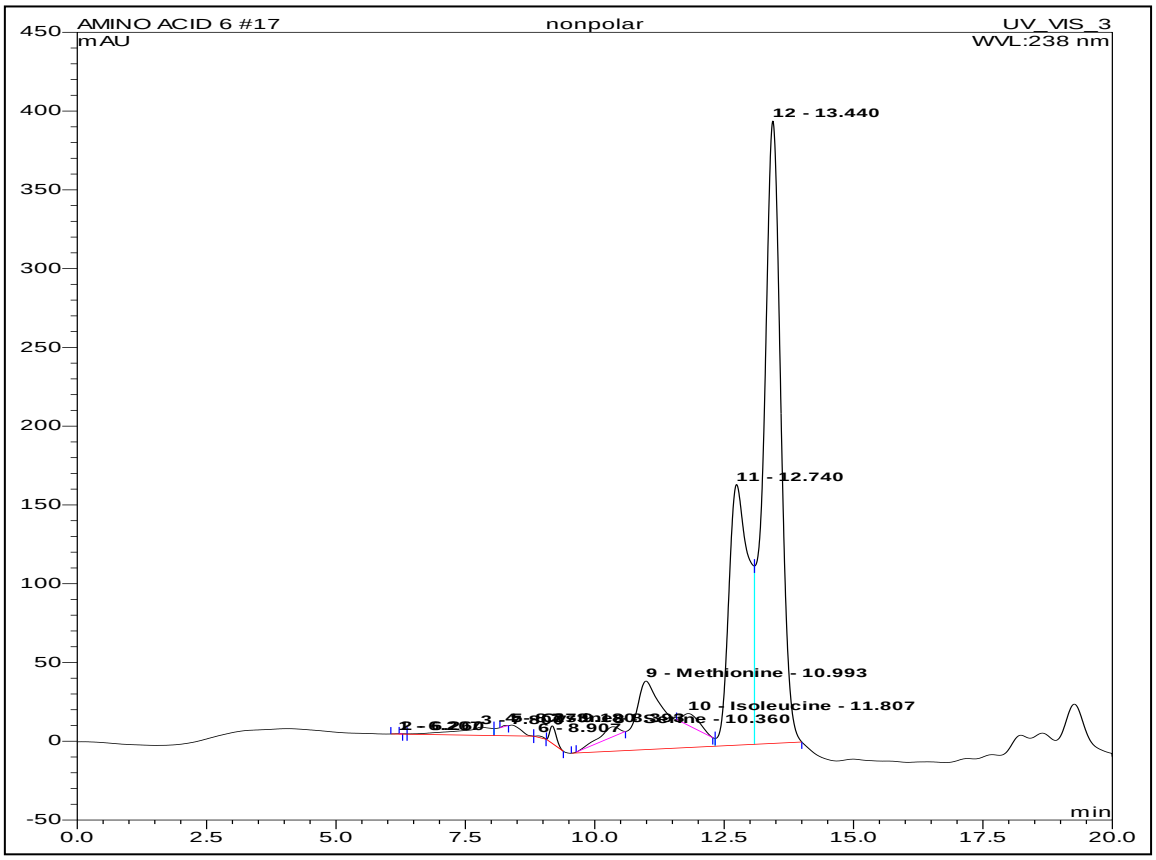


FIG : 8.0 HPLC OF AMINOACID STANDARDS

Amino acids	mg/l	Area (mau)	Retention time
Asparagine	0.04mg	10.702	7.34
Aspartic acid	0.04 mg	10.66	7.77
Cystine	0.24	14.55	8.44
Serine	0.05mg	5.290	10.35
Glycine	0.06mg	0.174	11.55
Methionine	0.1mg	60.299	10.96
Valine	0.3mg	66.387	9.14
Isoleucine	0.1mg	4.46	11.93
Phenylalanine	0.2mg	254.55	13.45
Alanine	0.05mg	217.63	13.36
Tyrosine	0.5mg	1432.699	26.85
Glutamic acid	0.05mg	189	10.92,11.39
Glutamine	0.03 mg	3.95	10.21
histidine	0.5mg	519.6	16.47
Proline	0.05mg	7	11.90

Table-1: Amino acid standards with retention time

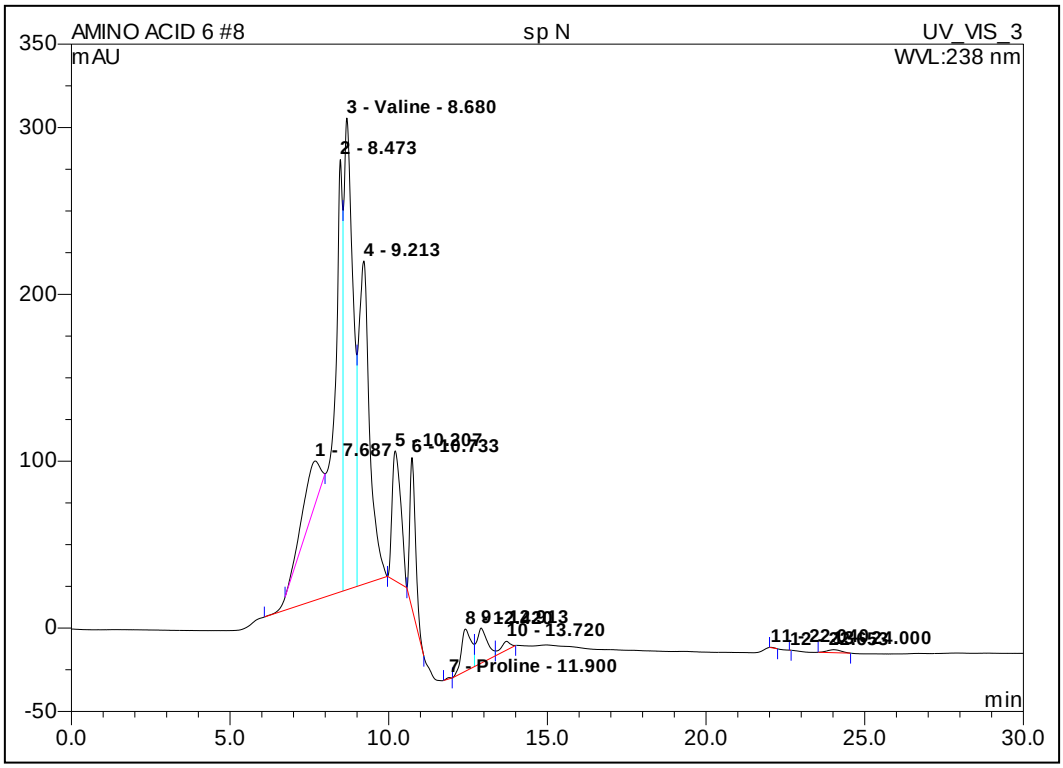


FIG :9.0 AMINOACID FINGER PRINT OF S. MAXIMA EXPOSED TO CONTROL (Endogenous aminoacids)

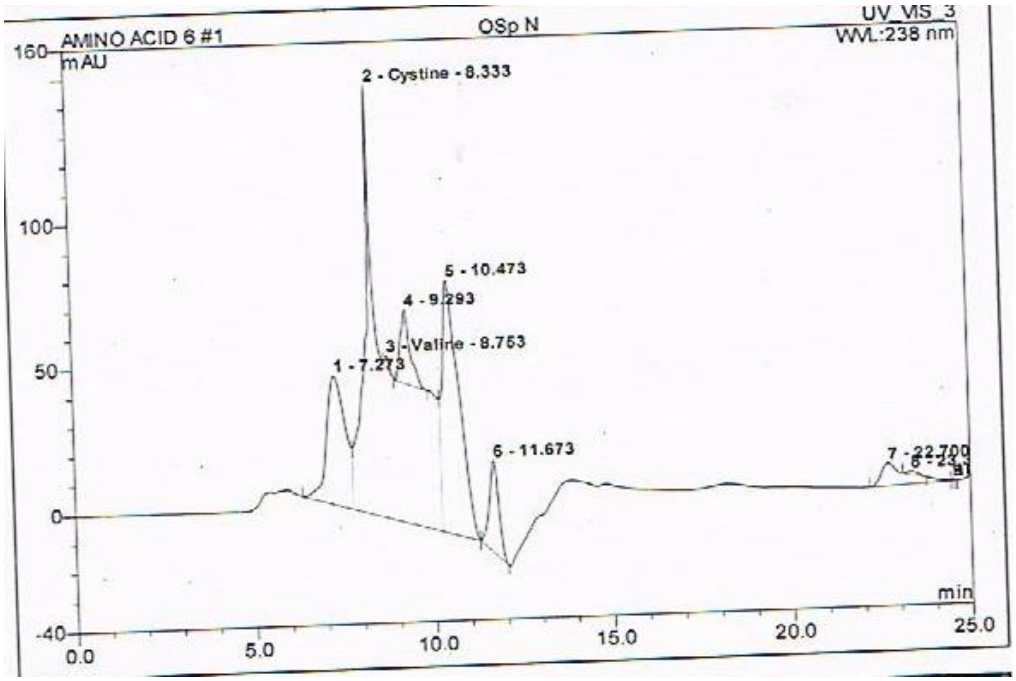


FIG :9.0 (b) AMINOACID FINGER PRINT OF S. MAXIMA EXPOSED TO CONTROL (cell wall aminoacids)

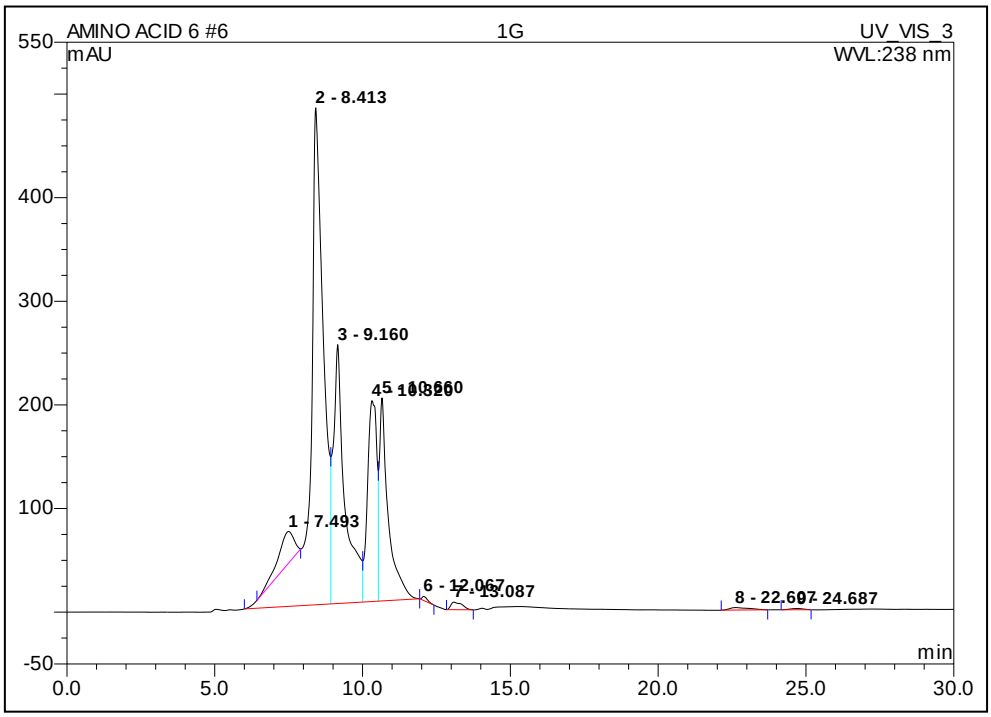


FIG:9.1 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 1G EMF (Intracellular)

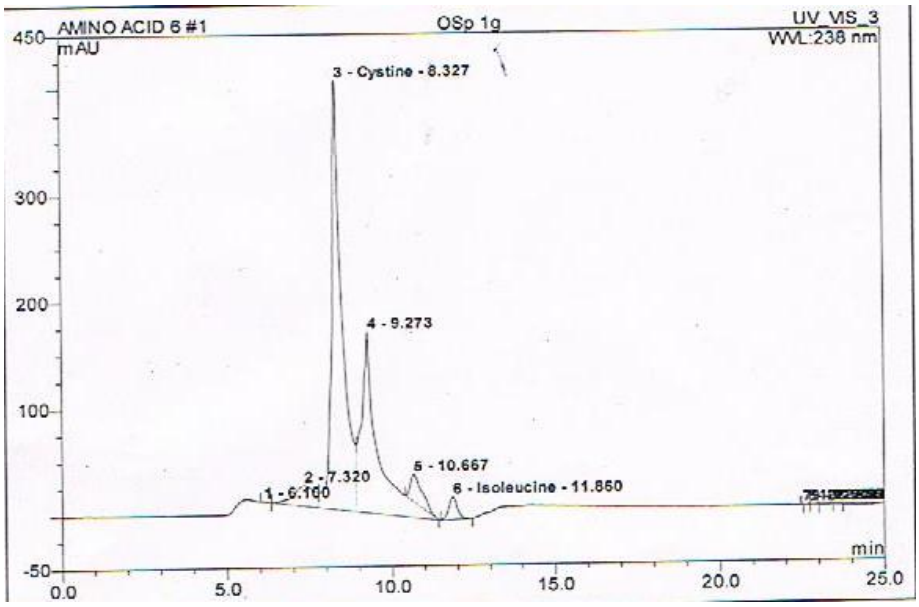


FIG:9.1 (b) AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 1G EMF (cell wall)

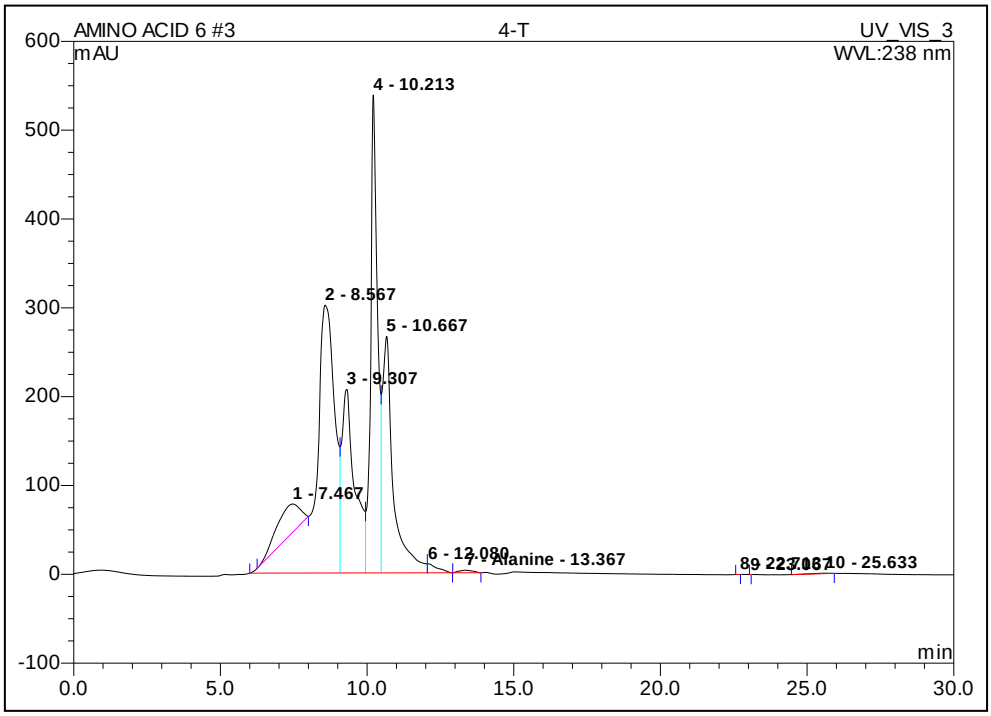


FIG: 9.2 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 400 G EMF (Intracellular)

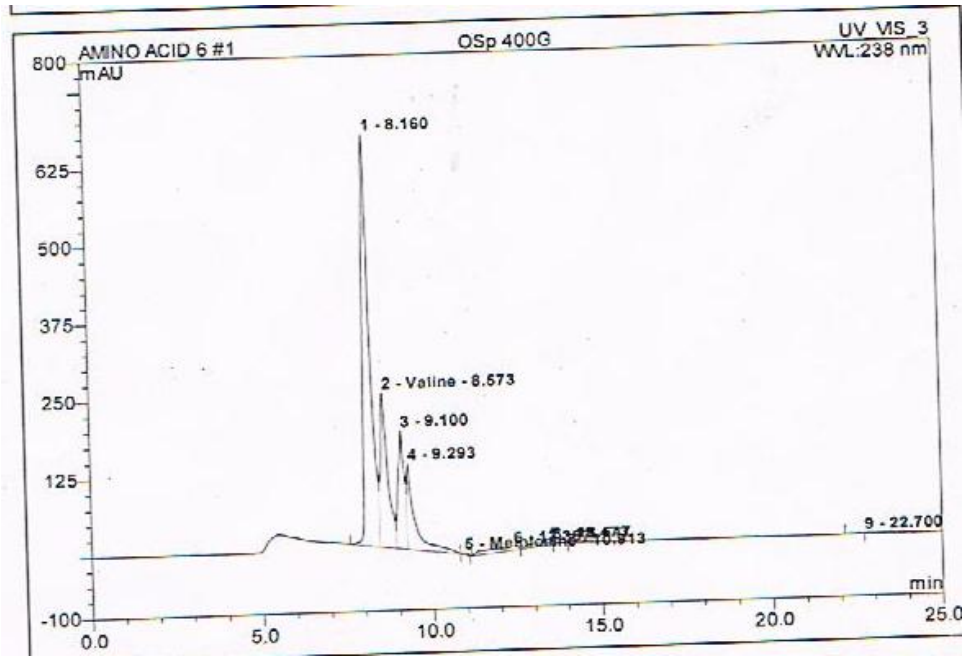


FIG: 9.2 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 400 G EMF (Cell wall)

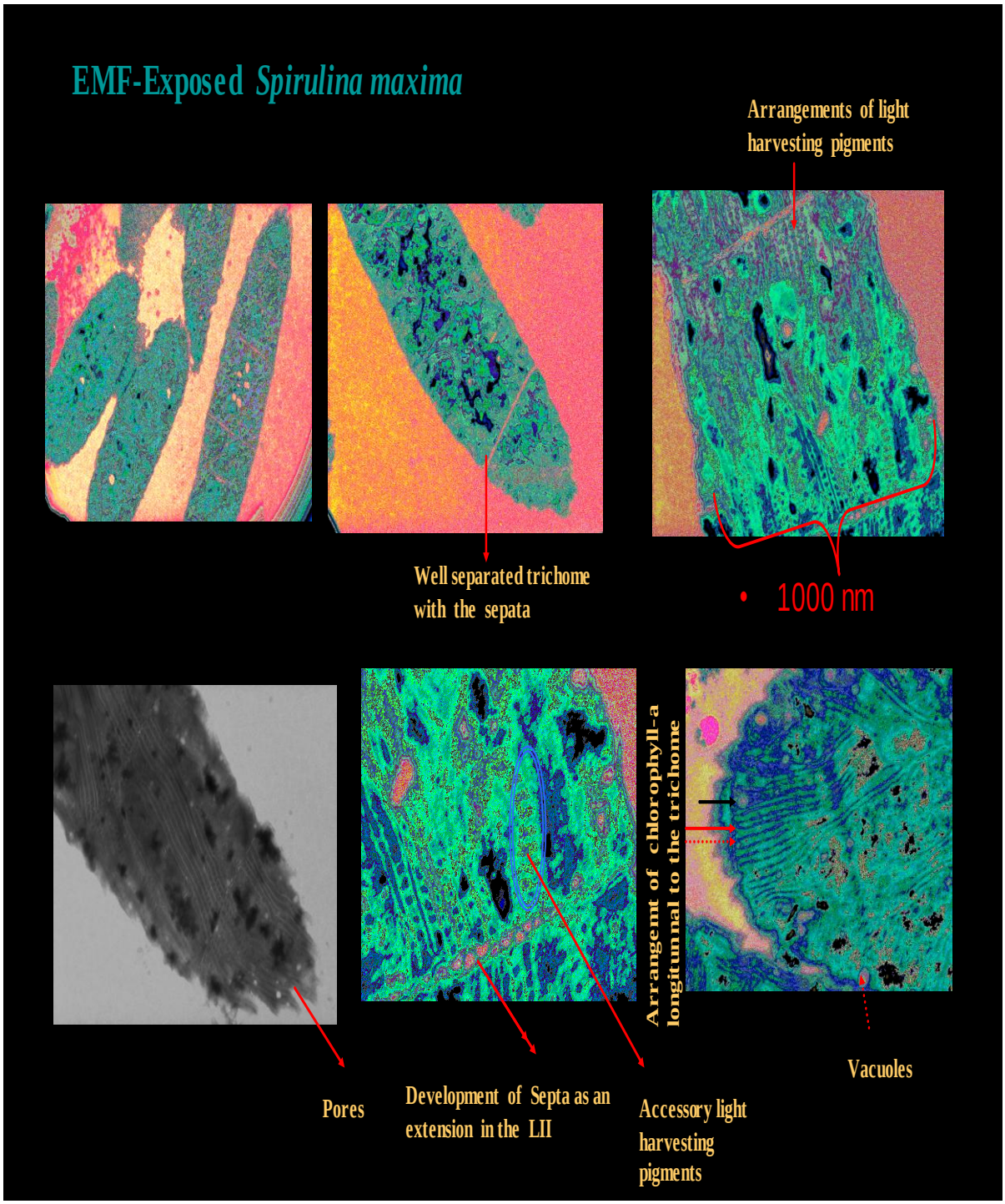


Fig 10 Transmission electron micrograph of *S. maxima* exposed to EMF
Magnified to 40,000 X – 60,000 X (40- 60 HT)

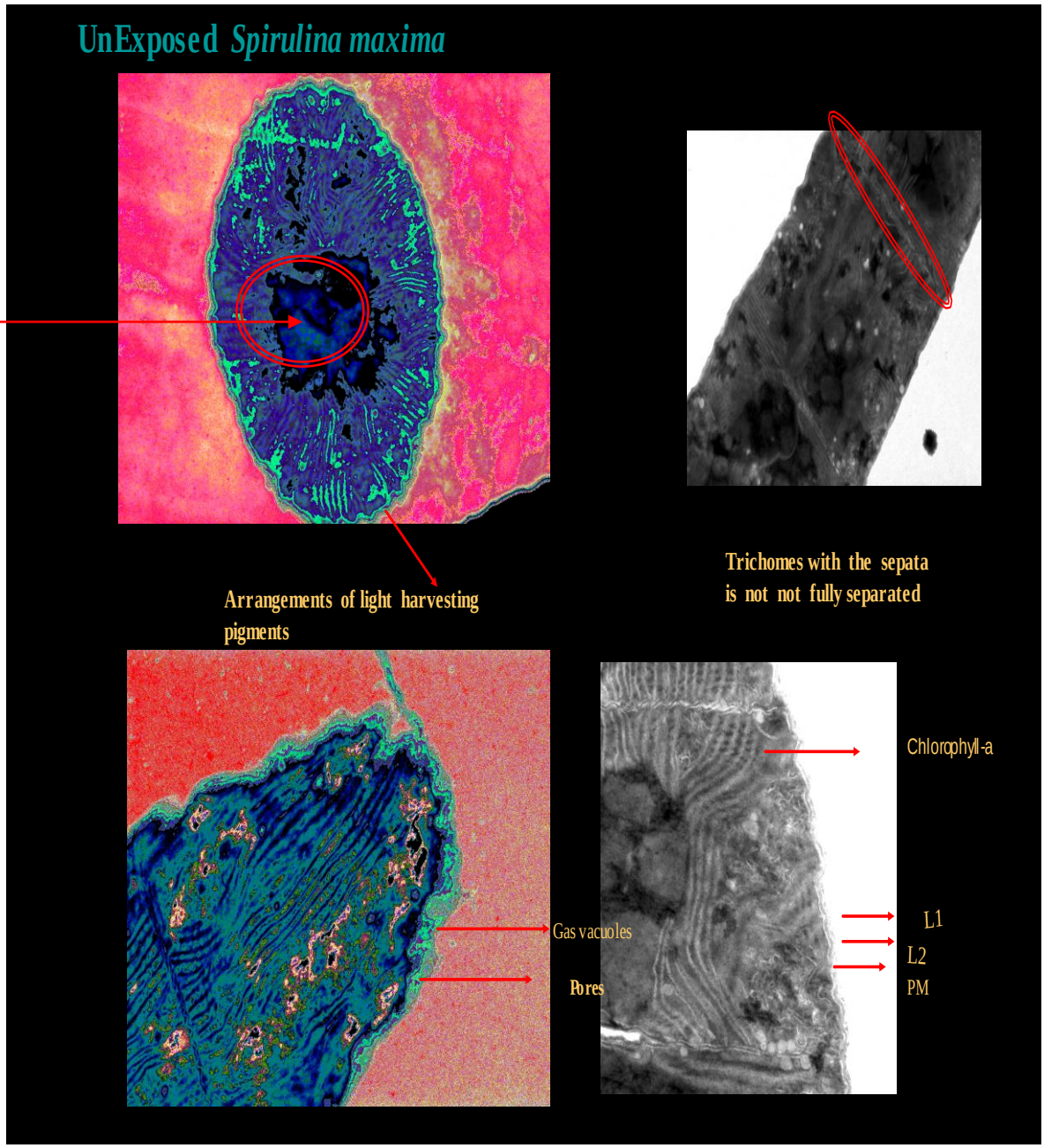


Fig 11 Transmission electron micrograph of *S. maxima* exposed to EMF
Magnified to 40,000 X – 60,000 X (40- 60 HT)

Screening of antidiabetic activity of *Spirulina maxima* in mild diabetic wistar rats:

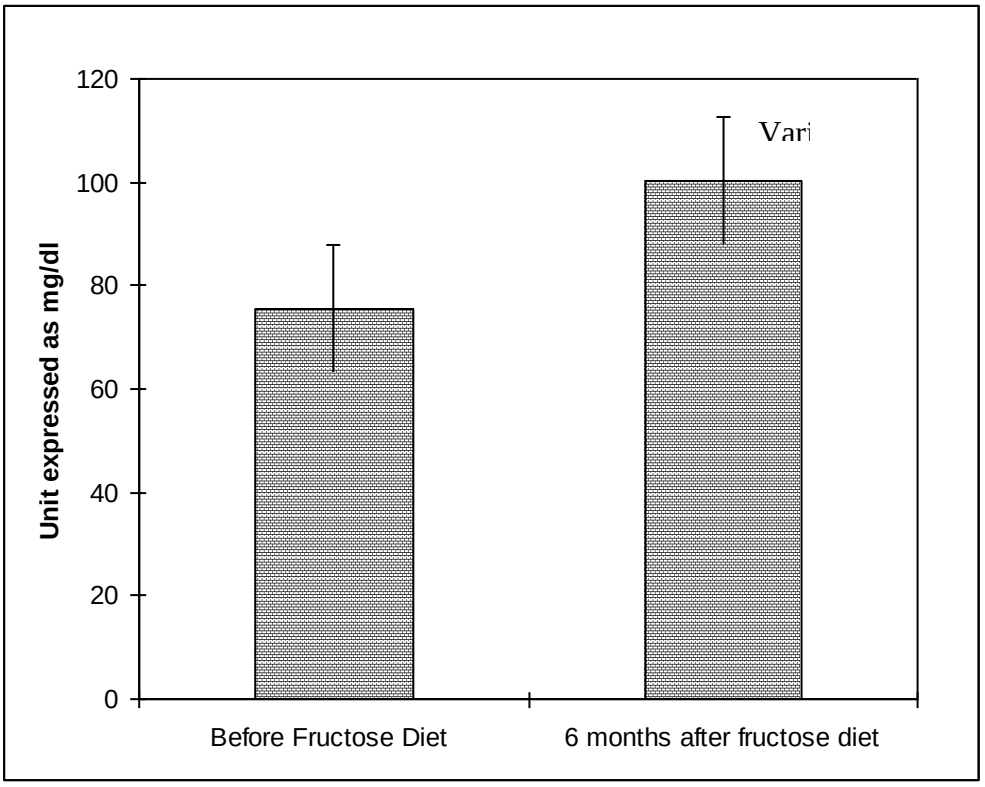


Figure (12) - Fasting blood glucose levels of rats kept on 72% Fructose rich diet.

Units expressed as mg/dl.

The values were expressed as mean ± SEM of n=5.

* statistically significant differences compared with before fructose diet ($P < 0.05$ one way ANOVA - Bonferroni test) calculated using the software Originpro Version 8.1.

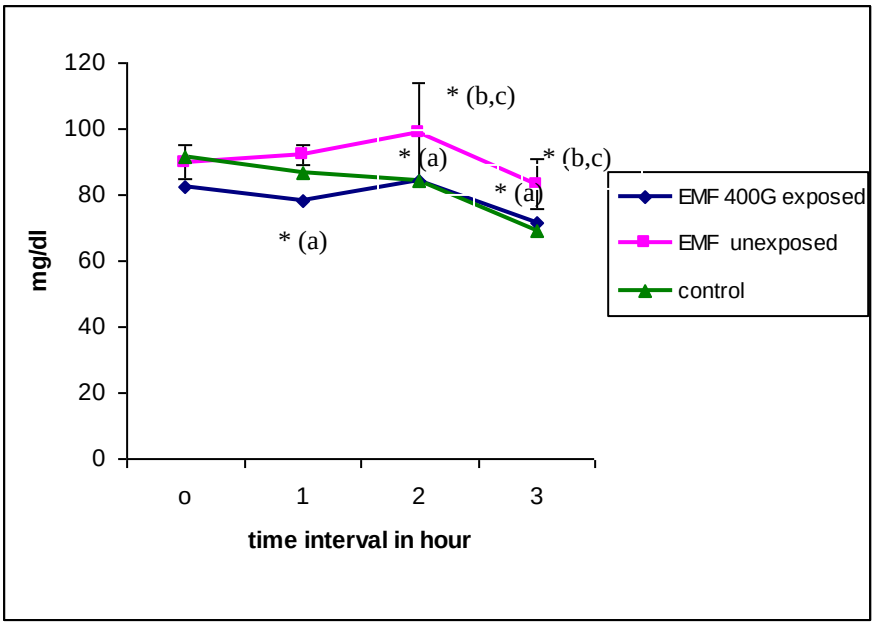
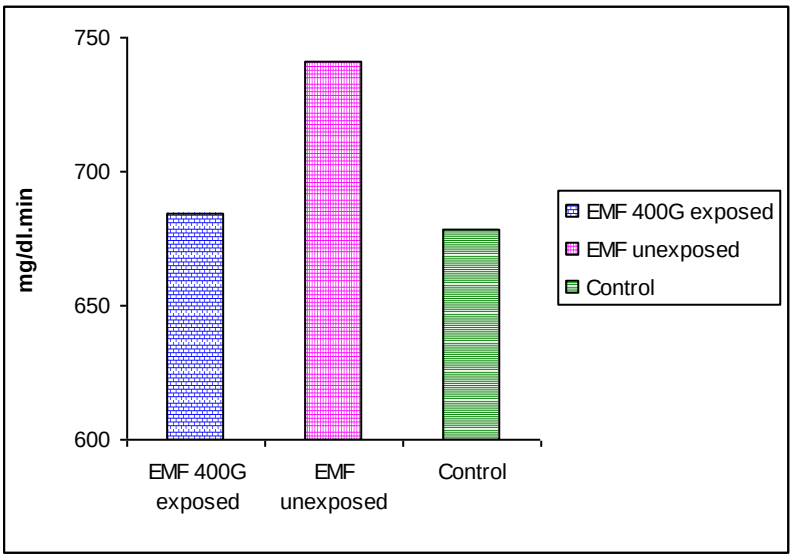


Figure (13) - *S. maxima* administration in fasted normal animals

Units expressed as mg/dl
The values were expressed as Mean ±S.E, n=2.
Changes in blood glucose levels (a) hourly blood glucose levels in EMF unexposed (b) hourly blood glucose levels in normal control (c) hourly blood glucose levels in EMF exposed (400 G), * statistically significant change (P< 0.05 one way ANOVA - Bonferroni test) calculated using the software Originpro Version 8.1.



Units expressed as mg/dl.min
Figure (14) - Area under curve graph of normal fasting animals.

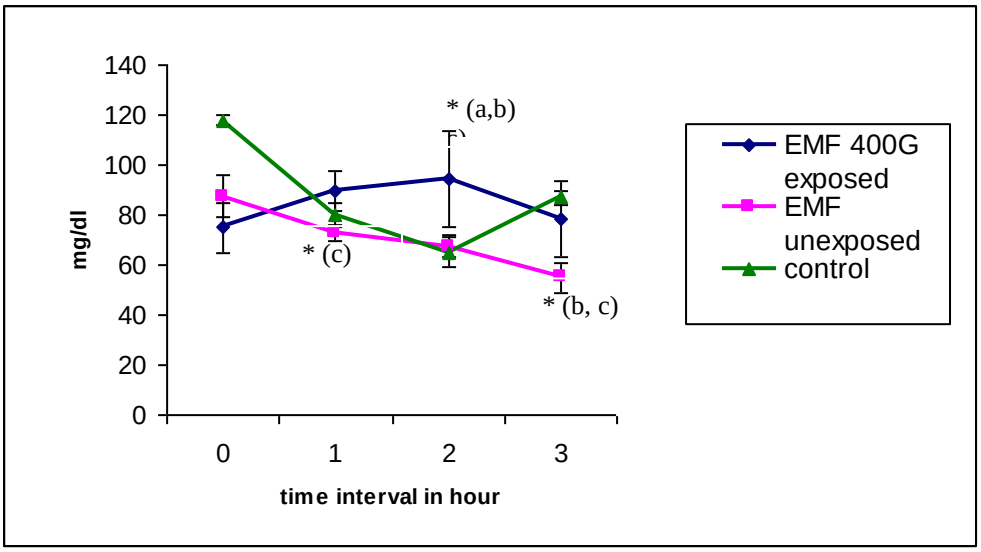


Figure (15) - *S. maxima* administration in diabetic fasted animals.

Units expressed as mg/dl
The values were expressed as Mean ±S.E, n=2.
Changes in blood glucose levels (a) hourly blood glucose levels in EMF unexposed (b) hourly blood glucose levels in diabetic control (c) hourly blood glucose levels in EMF exposed (400 G) * statistically significant change (P< 0.05 one way ANOVA - Bonferroni test) calculated using the software Originpro Version 8.1

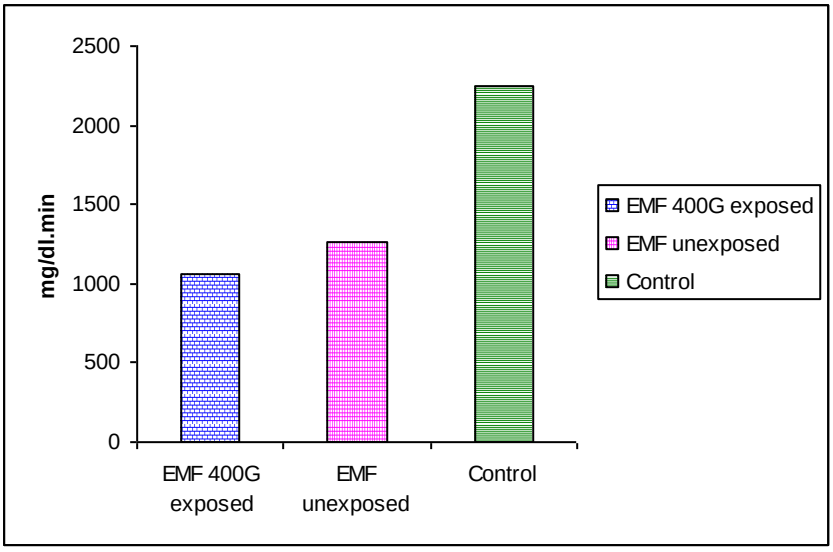


Figure (16) - Area under curve graph of diabetic fasting animals.
Units expressed as mg/dl.min

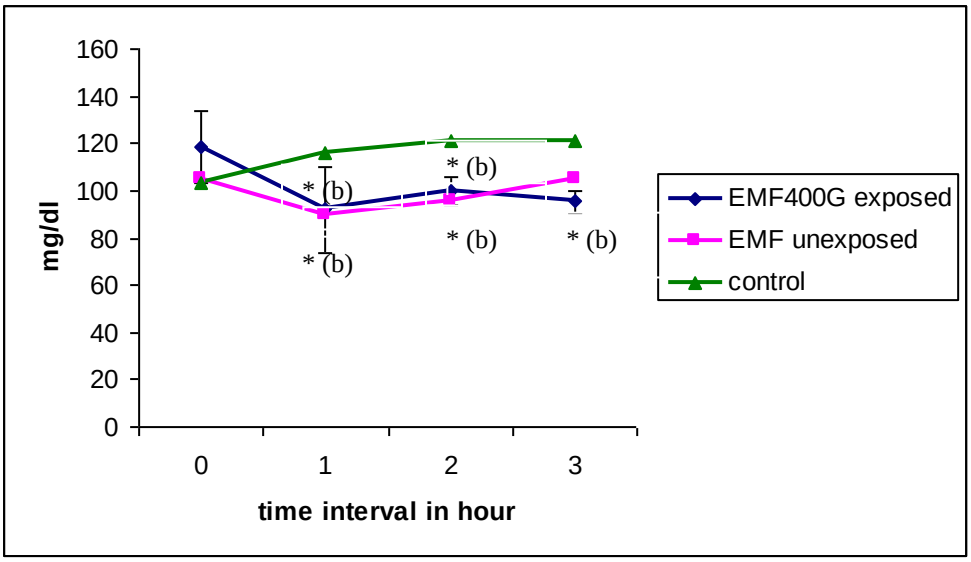


Figure (17) – ***S. maxima*** **administration in non-fasted normal animals.**

Units expressed as mg/dl
The values were expressed as Mean ±S.E, n=2.
Changes in blood glucose levels (a) hourly blood glucose levels in EMF unexposed (b) hourly blood glucose levels in normal control (c) hourly blood glucose levels in EMF exposed (400 G) * statistically significant change (P< 0.05 one way ANOVA - Bonferroni test) calculated using the software Originpro Version 8.1.

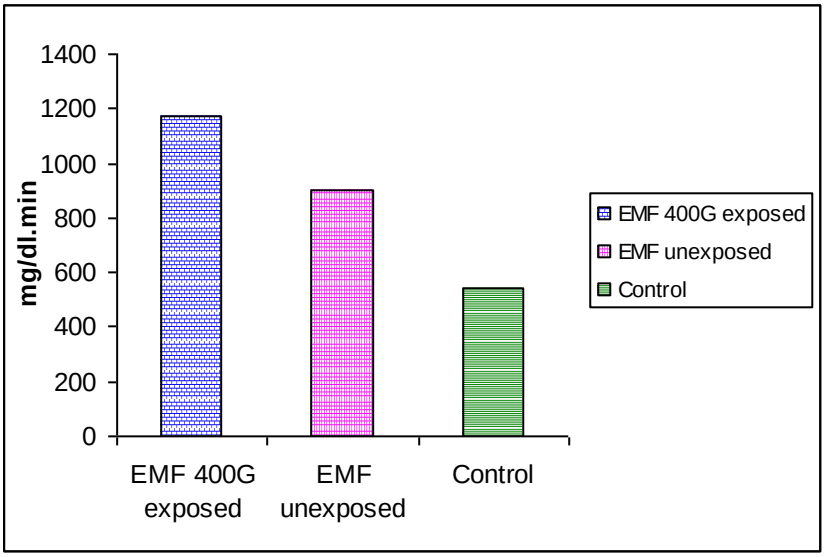


Figure (18) - Area under curve graph of normal non-fasting animals.
Units expressed as mg/dl.min

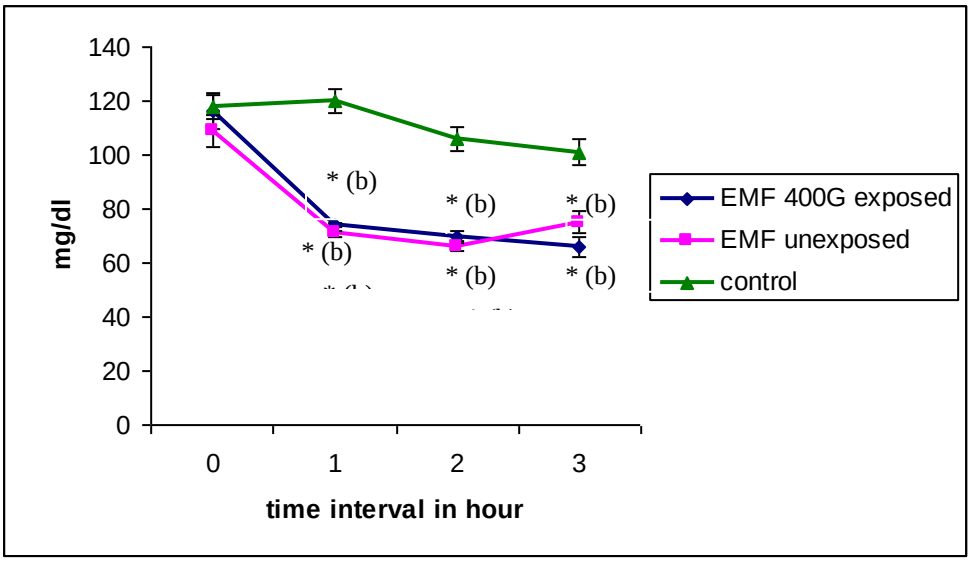


Figure (19) – *S. maxima* administration in diabetic non-fasted animals.

Units expressed as mg/dl
The values were expressed as Mean ±S.E, n=2.
Changes in blood glucose levels (a) hourly blood glucose levels in EMF unexposed (b) hourly blood glucose levels in diabetic control (c) hourly blood glucose levels in EMF exposed (400 G) * statistically significant change (P< 0.05 one way ANOVA - Bonferroni test) calculated using the software Originpro Version 8.1

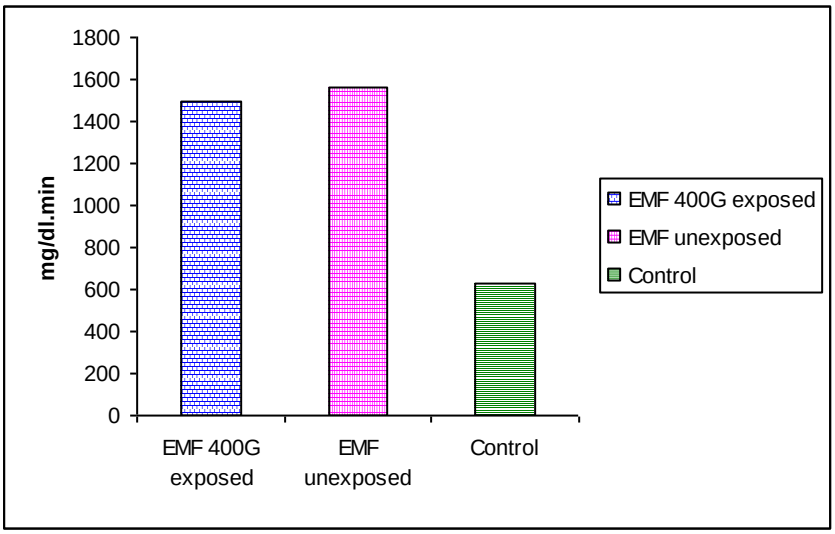


Figure (20) - Area under curve graph of diabetic non-fasting animals.

Units expressed as mg/dl.min

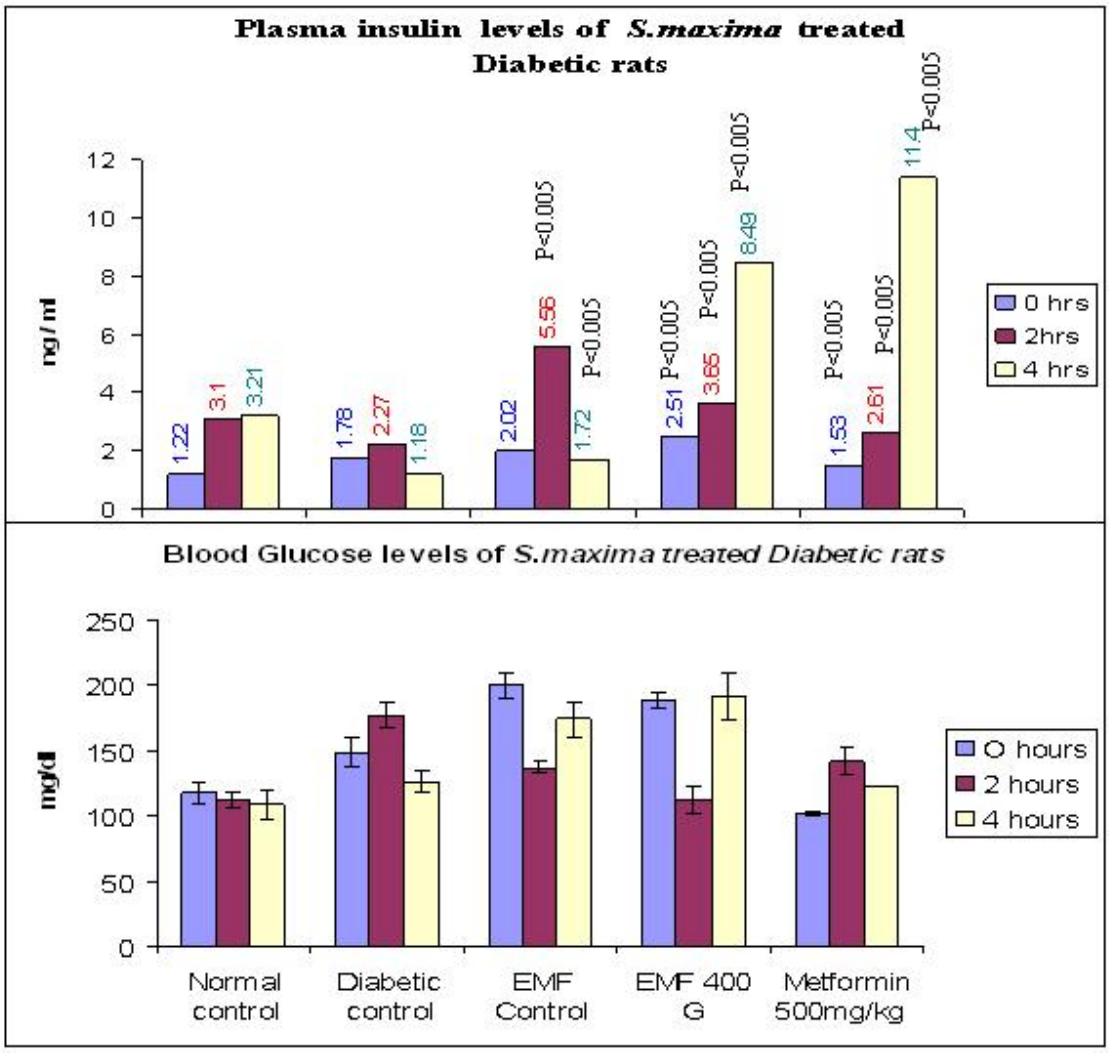


Figure (21)- Plasma Blood glucose levels and Insulin levels

Units expressed for Blood glucose ; mg/dl

Units expressed for Insulin levels ; ng/ml

Cultivation of S. maxima Exposed to EMF and High light intensity

Different studies have been focused on in enriching the growth and metabolites of *Spirulina maxima*, the optimized conditions enhanced the only the

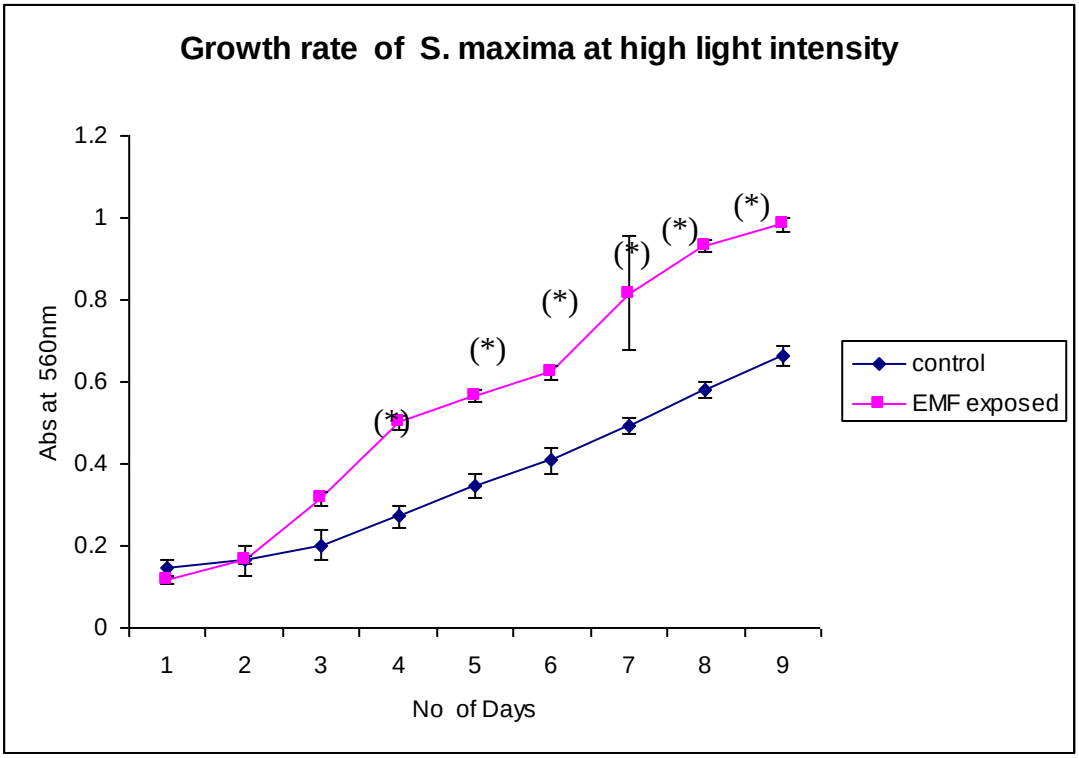


Fig :21 Growth rate of S. maxima exposed to high light intensity
Light intensity 10000 lumens
EMF exposed : 1 Gauss intensity
The unit expressed in absorbance

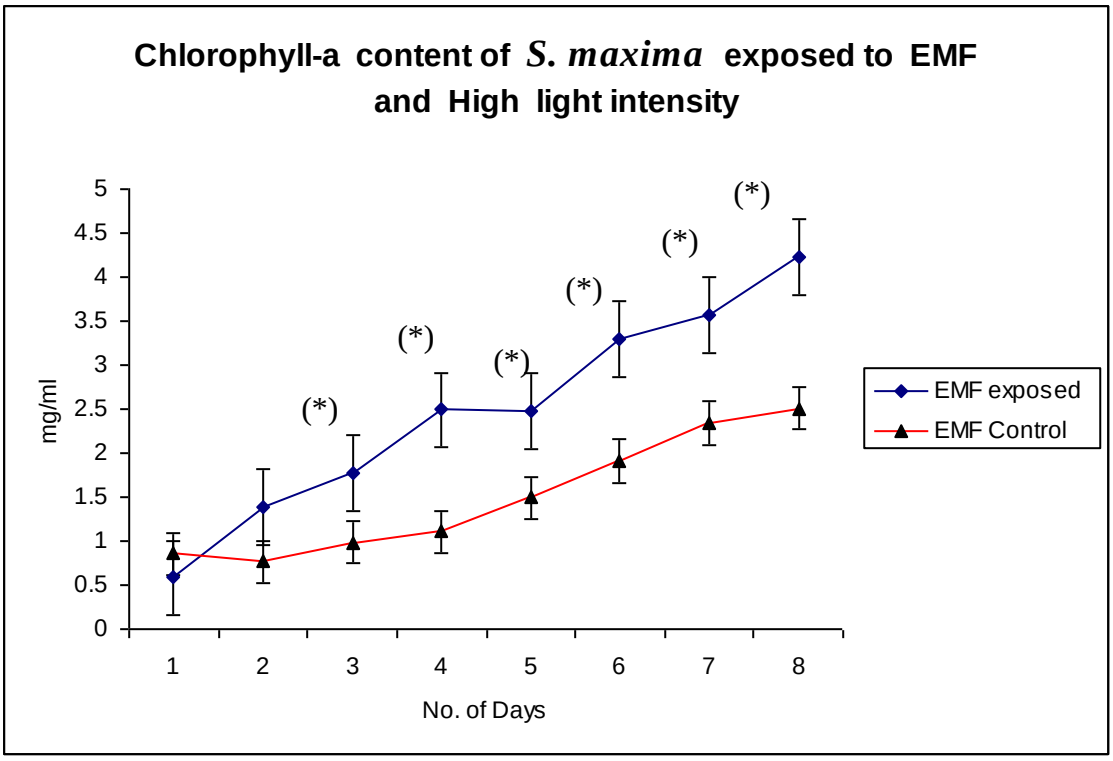


Fig :22 Chlorophyll-a CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed in 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the P<0.05 level of Significance with respect to the control

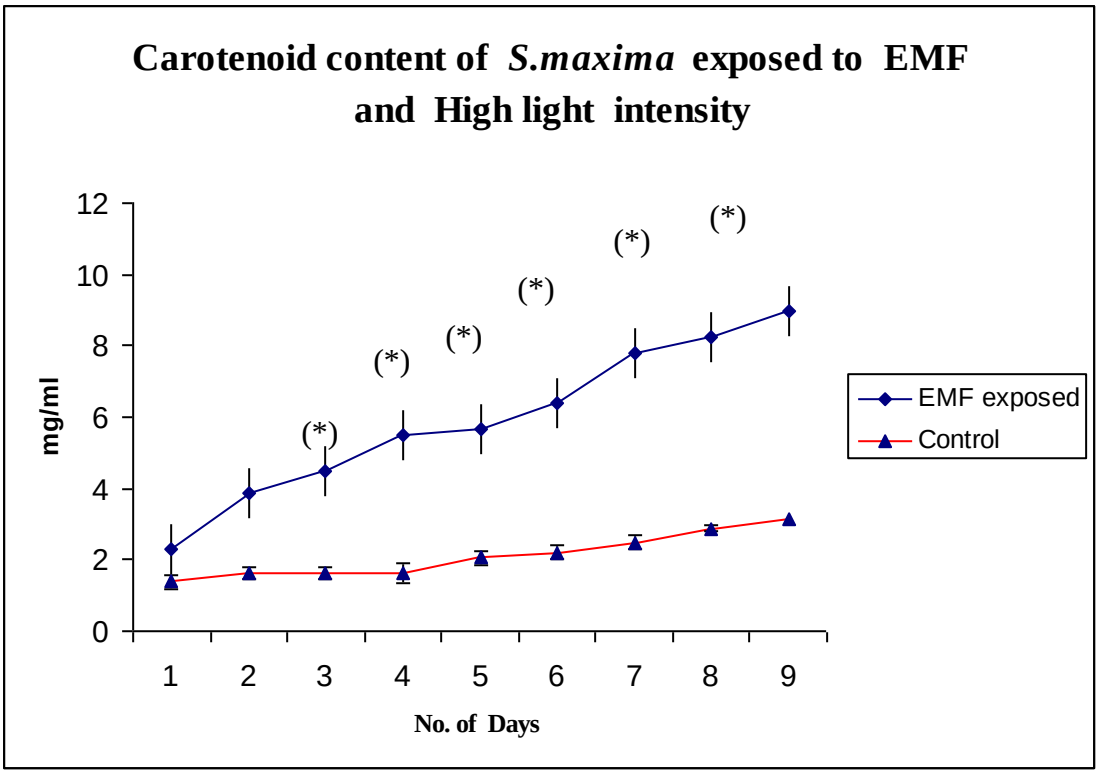


Fig :23 Carotenoid CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed in 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the $P<0.05$ level of Significance with respect to the control

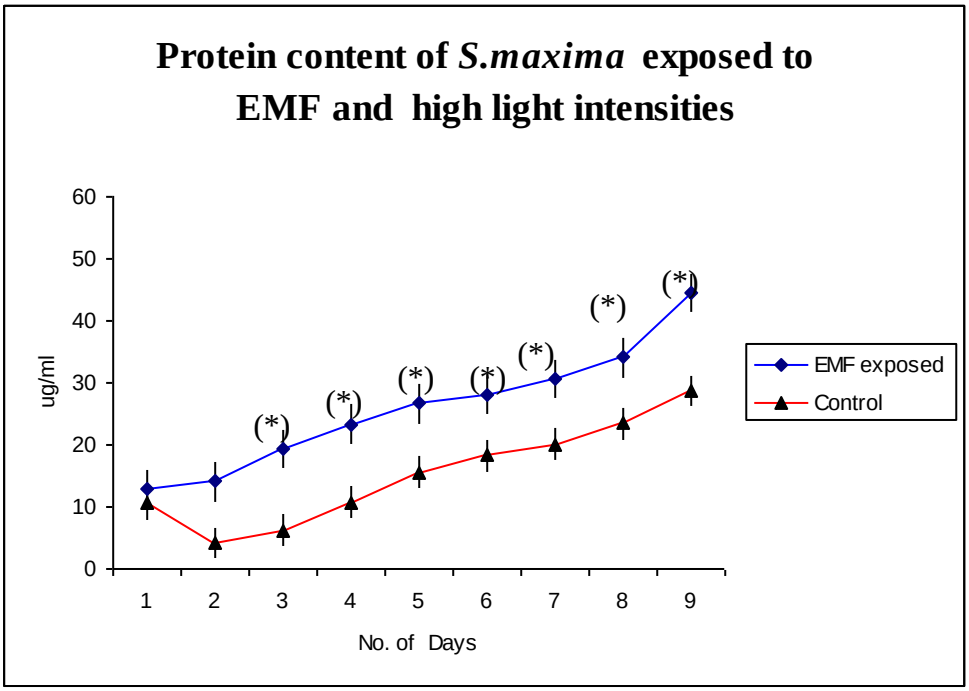


Fig :24 TOTAL PROTEIN CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed in 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the $P<0.05$ level of Significance with respect to the control

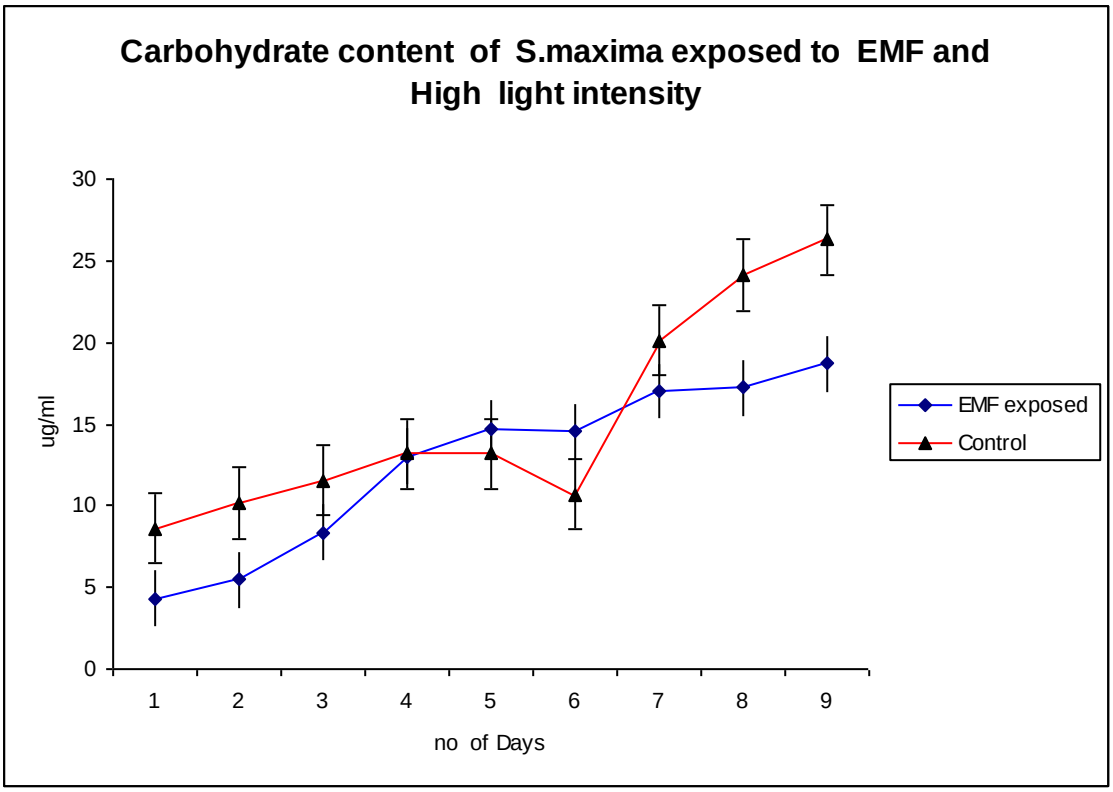


Fig :25 Carbohydrate CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed in 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the P<0.05 level of Significance with respect to the control

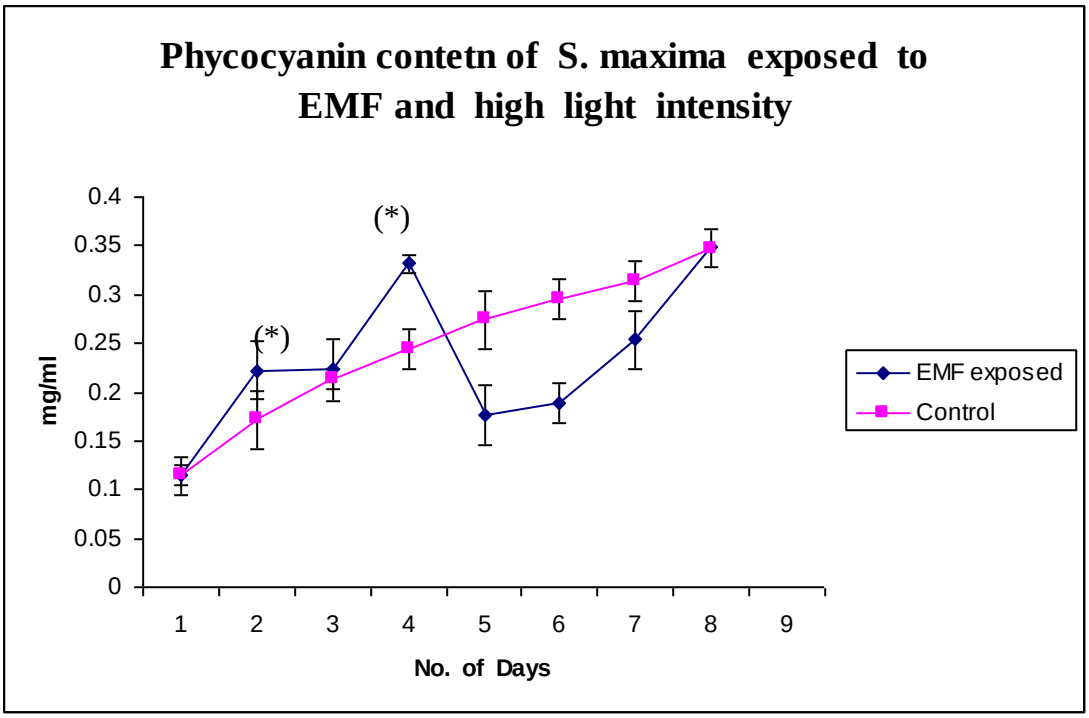


Fig :26 Phycocyanin CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed in 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the $P<0.05$ level of Significance with respect to the control

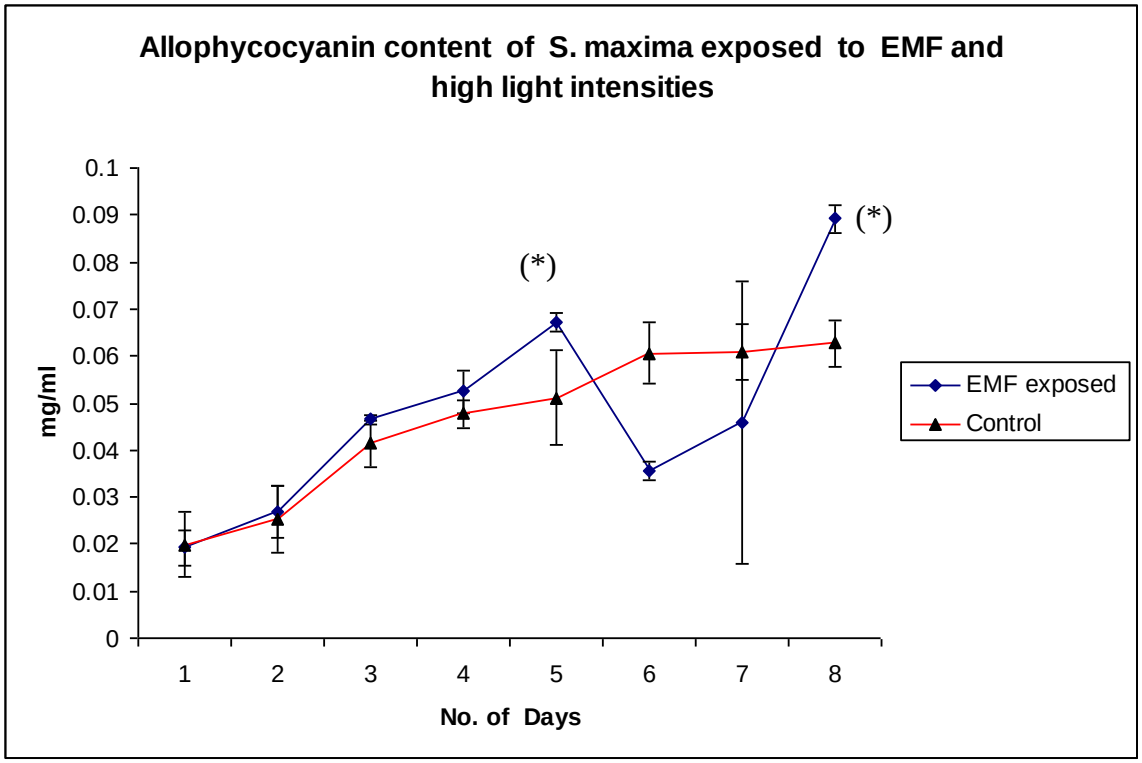


Fig :26 ALLOPHYCOCYANIN CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed is 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the $P<0.05$ level of Significance with respect to the control

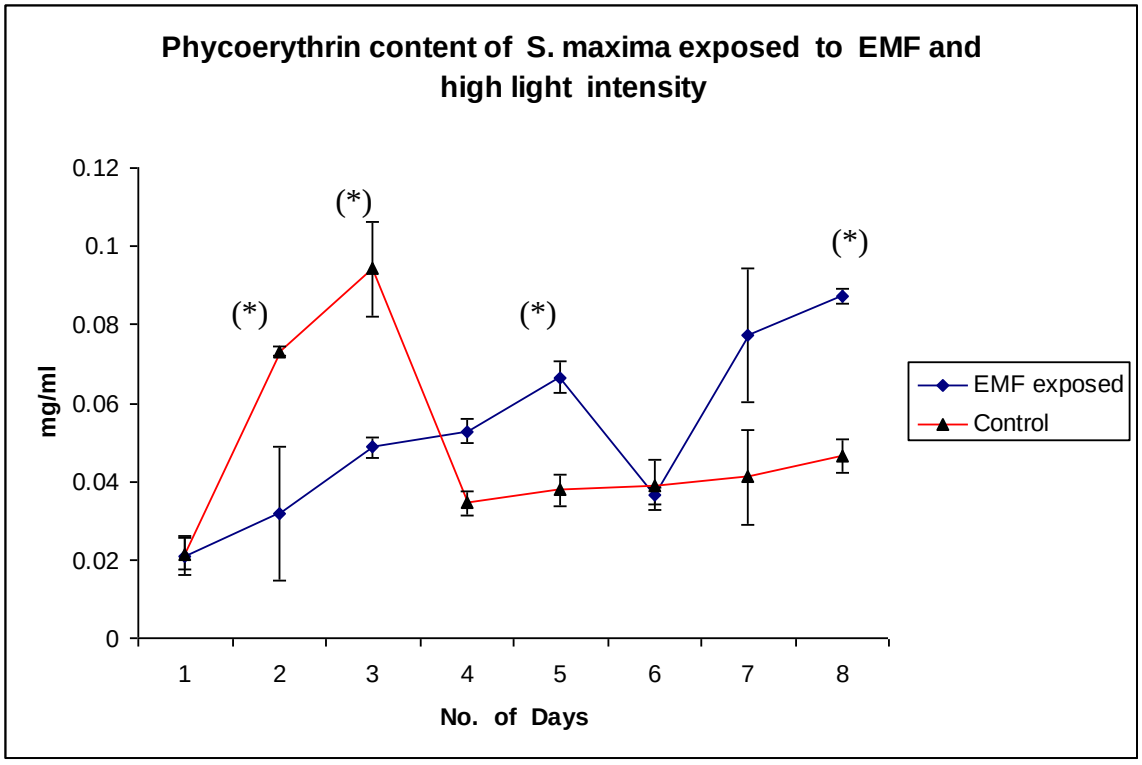


Fig :26 PHYCOERYTHRIN CONTENT OF *S. maxima* CULTIVATED IN AIRLIFT PHOTOBIOREACTOR UNDER EMF EXPOSURE and High light intensity

The total protein content unit expressed as mg/ml
The data values expressed as Mean+S.E

The light inensity exposed in 10000 lumens

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the $P<0.05$ level of Significance with respect to the control

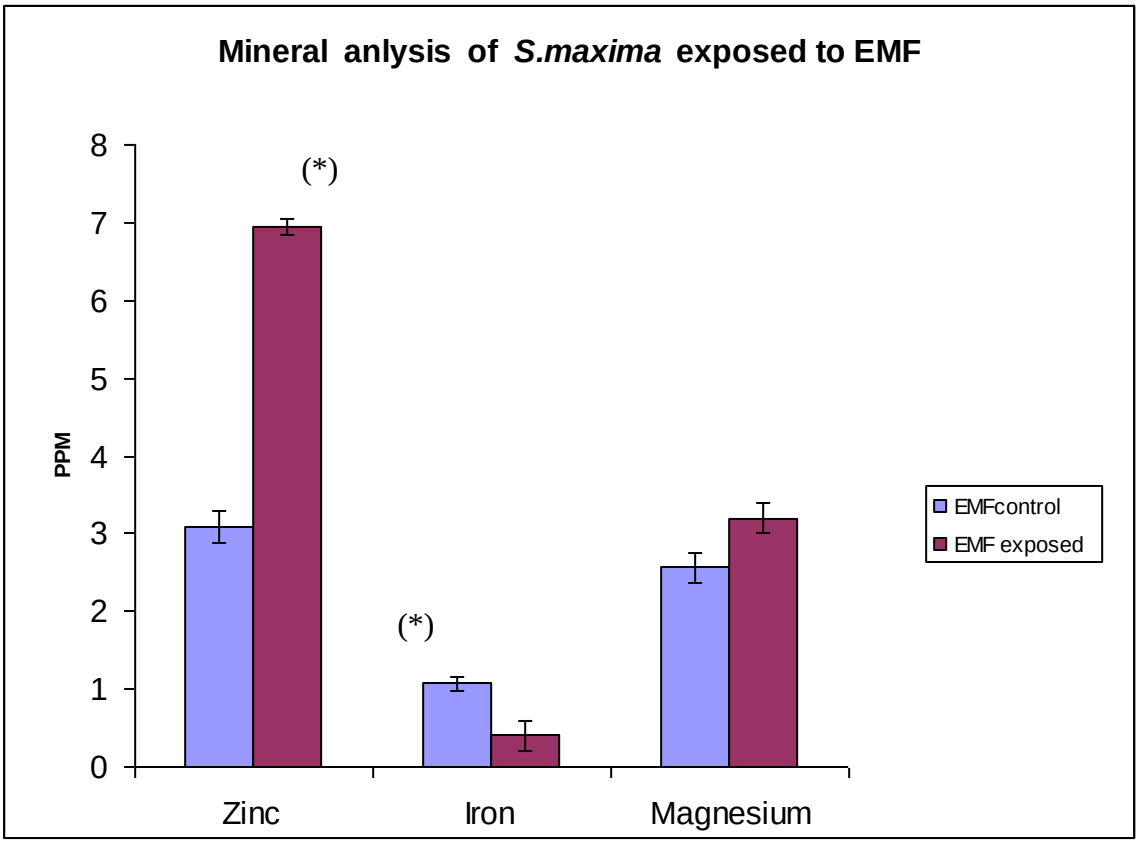


Fig: 27 Mineral anlysis of S. maxima exposed to EMF

Minerals expressed as Parts per million

EMF intensity exposed : 1 Guass

Student T- test was performed to anlyze the stastically significant difference between the control and exposed groups

(*) indicate the $P<0.05$ level of Significance with respect to the control

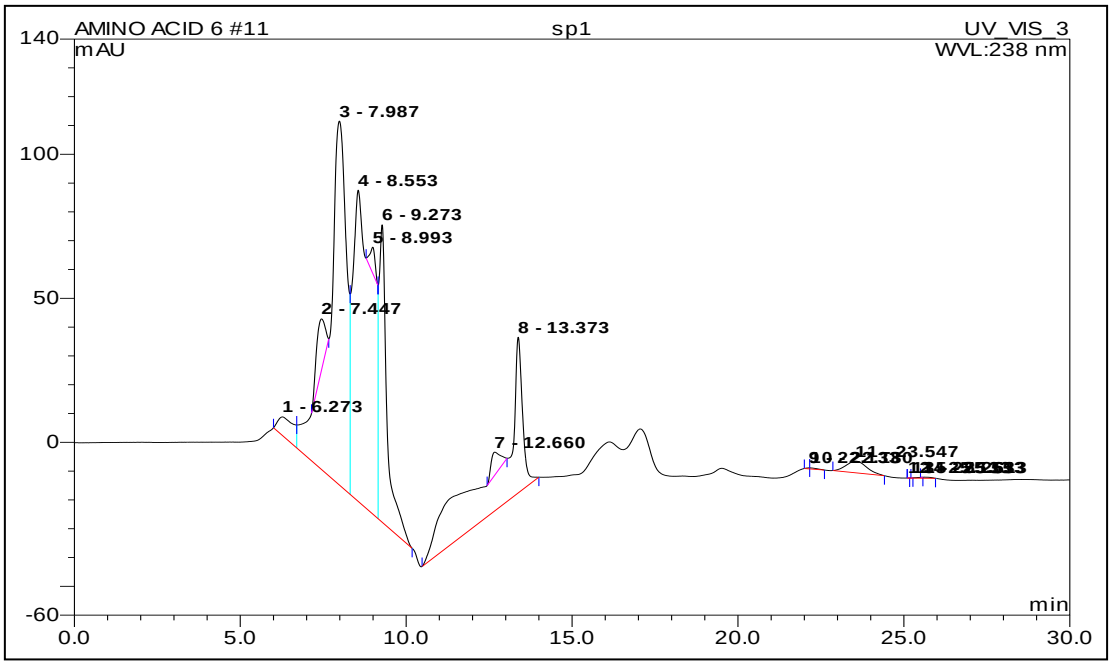


FIG- 28: AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO CONTROL(Endogenous)

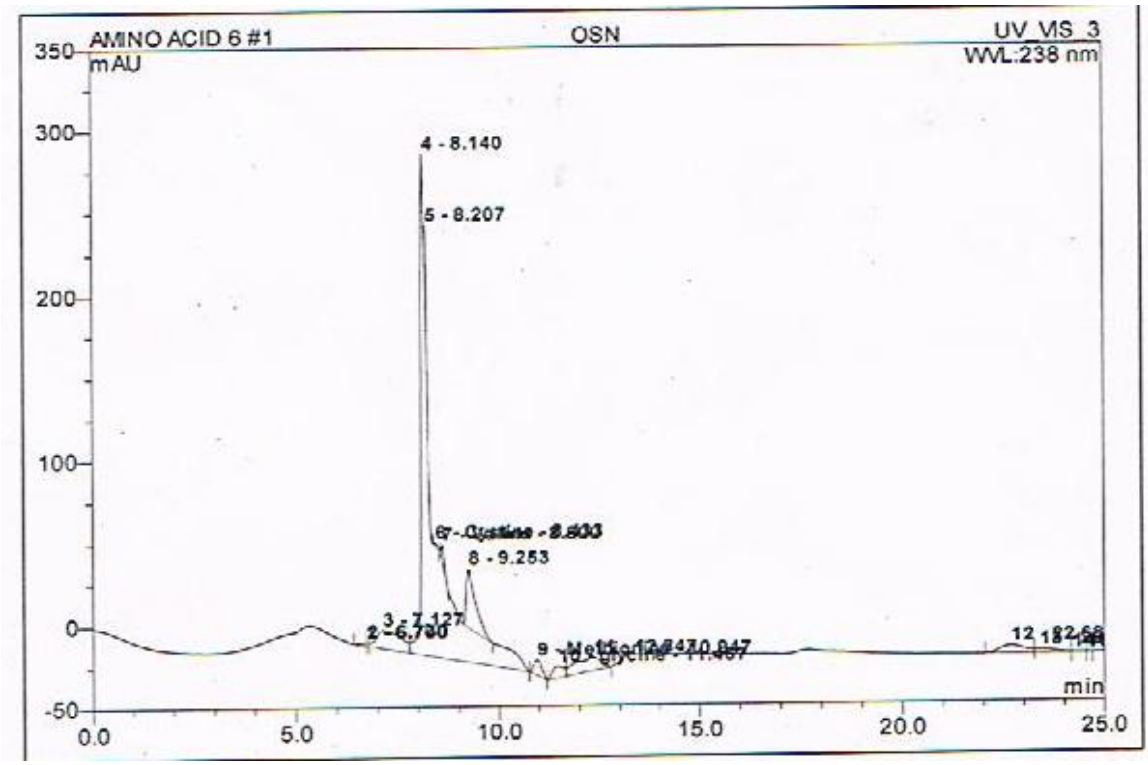


FIG- 28 (b): AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO CONTROL(Exogenous)

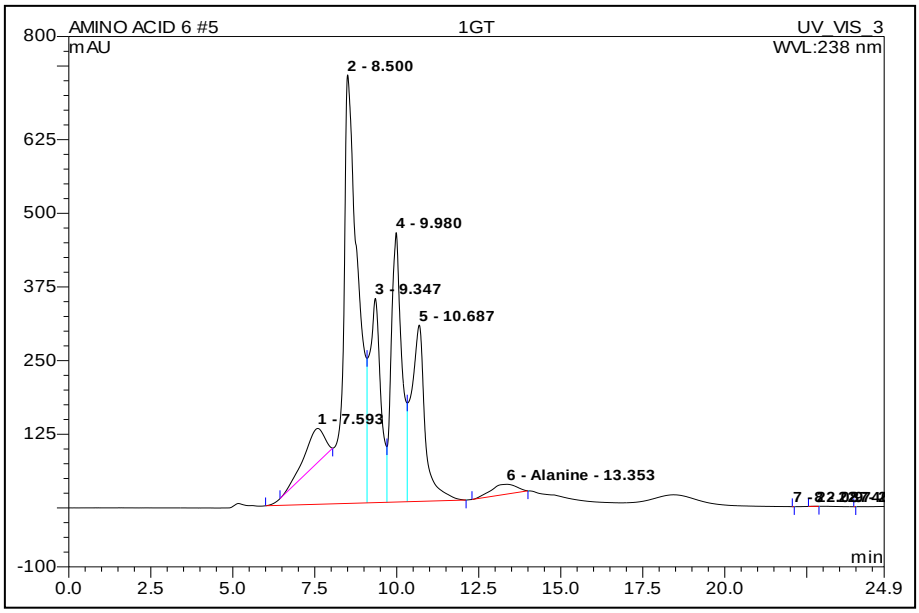


FIG 29 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 1G EMF AND HIGH LIGHT INTENSITY (Endogenous).

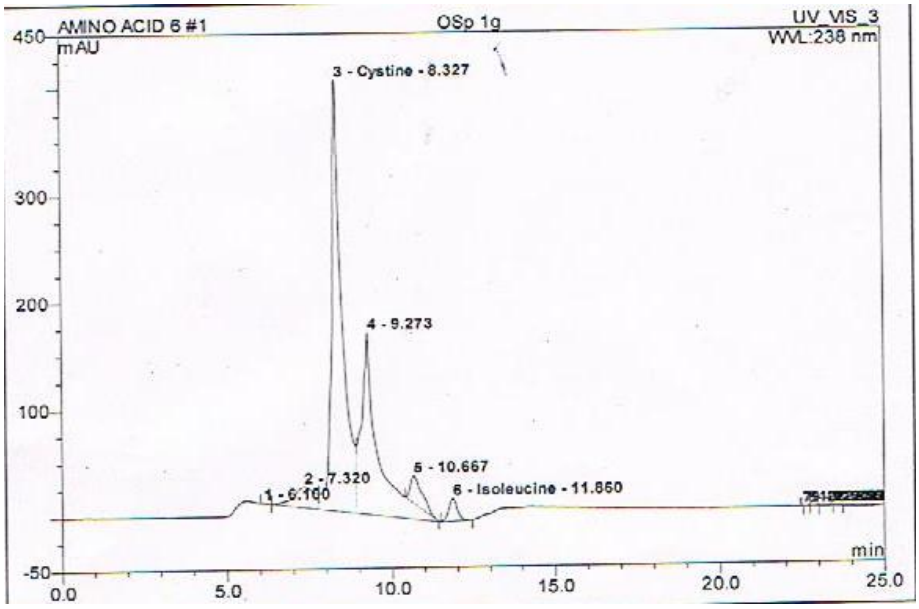


FIG- 29 (b) AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 1G EMF AND HIGH LIGHT INTENSITY (Exogenous).

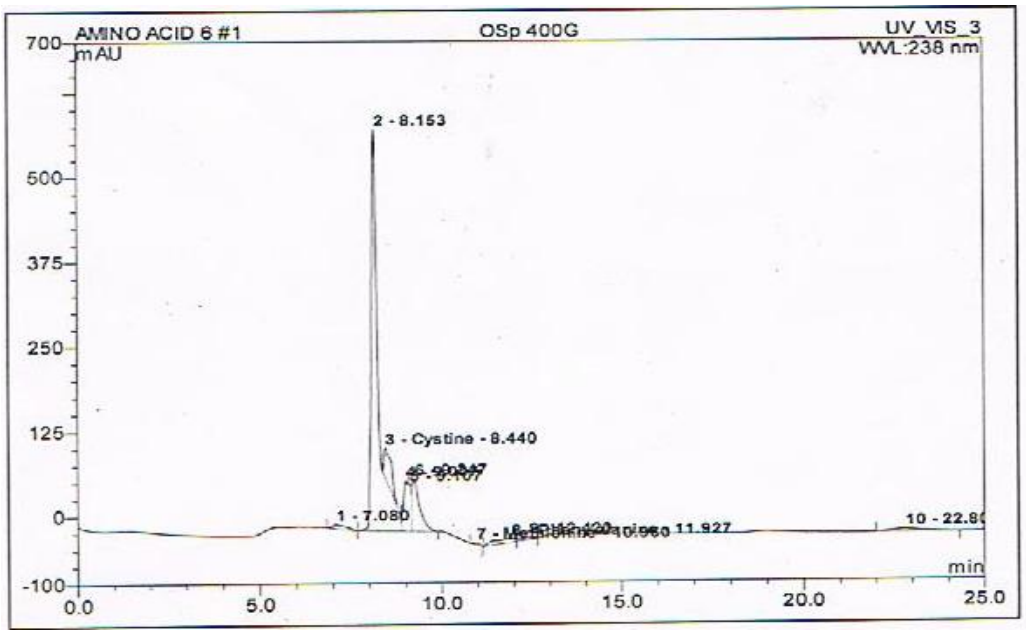


FIG- 30 AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 400 G EMF AND HIGH LIGHT INTENSITY.(Endogenous)

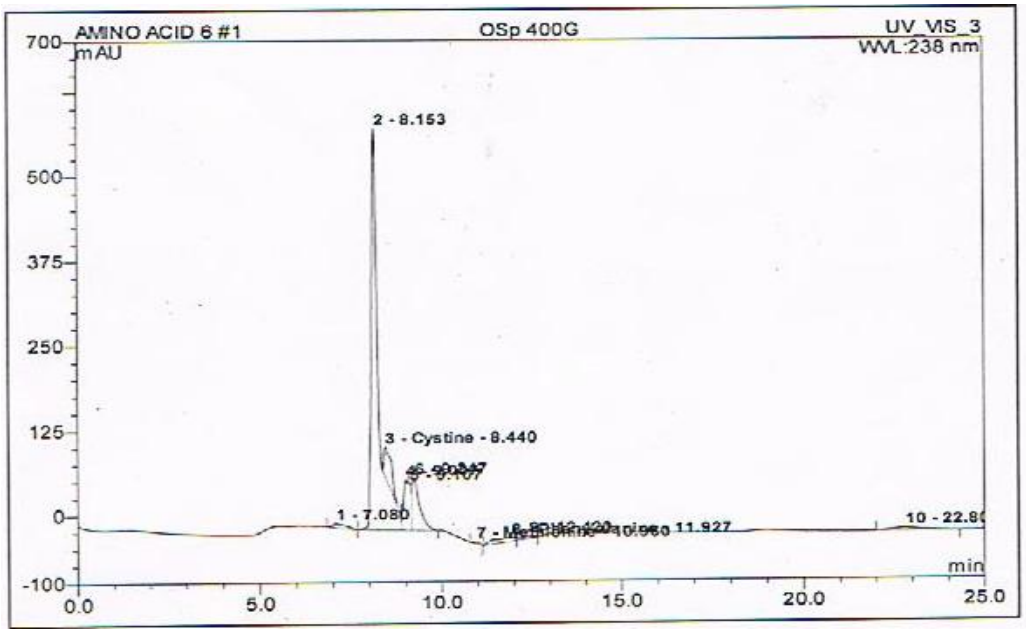


FIG- 30b. AMINO ACID FINGER PRINT OF S. MAXIMA EXPOSED TO 400 G EMF AND HIGH LIGHT INTENSITY.(Exogenous)

1 G EMF exposed	1 G EMF exposed at 5000 lumens	1 G EMF exposed at 10000 lumens	400 G EMF exposed at 5000 lumens	400 G EMF exposed at 10000 lumens	Control at 5000 lumens	Control At 10,000 lumens
Asparafine	+	+		+	+	+
Cystine	+	+	+	+	+	+
Valine	+	+	+	+	+	+
Methionine	+	+	+	+	+	-
Isoleucine	+	+	-	-	-	-
Phenylalanine	-	-	+	+	-	-
glycine	-	-	-	-	+	+
serine	-	-	-	-	-	+
	-	-	-	-	-	-
	-	-	-	-	-	-

Table -2 :Exogenous amino acid composition of *S. maxima*

1 G EMF exposed	1 G EMF exposed at 5000 lumens	1 G EMF exposed at 10000 lumens	400 G EMF exposed at 5000 lumens	400 G EMF exposed at 10000 lumens	Control at 5000 lumens	Control At 10,000 lumens
Asparafine	+	-	-	+	-	-
Cystine	+	+	+	+	+	+
Valine	+	+	-	+	+	+
Methionine	-	+	+	+	-	-
Isoleucine	-	+	+	-	-	-
Phenylalanine	-	-	+	+	+	+
glycine	-	-	+	+	-	-
serine	+	-	-	-	+	-
Alanine	+	+	+	-	-	-
Aspartic acid	+	+	+	-	+	+
Glutamic acid	+	+	+	+	-	+
Glutamine	-	+	+	+	-	+
Proline	-	+	+	-	-	+

Table-3 :Endogenous amino acid composition of *S. maxima*

Discussion

The present study was focused on the cultivation of *S. maxima* in airlift photobioreactor exposed to different electromagnetic intensities. The studies resulted in the increase in the growth rate of *S. maxima* in EMF 1 G exposure. Whereas a detrimental effect was observed when exposed to 400G. Similar study carried out by Hirano *et al*., 1998 revealed the maximum growth rate of *S. platensis*, when exposed to 100 G. The chlorophyll-a content and photosynthetic rate was also found to be maximum in 1 G exposed *S. maxima*, the results suggests that magnetic fields accelerate the light excitation of chlorophyll, reactions of electron transfer and conversion of light energy to chemical energy. The lipid content was found to decrease in 400 G exposed *S. maxima* similar was the observations of Hirano *et al* (1998), which revealed a reduction in the algal glyceroglycolipid content when exposed to 400 G intensities and above. The protein content was also found to decrease with EMF exposure, the effect of EMF on the protein is not widely studied and understood. However the growth of S-maxima was inhibited at 400 G & the sugar content decreased with increase in magnetic flux intensity. These are assumed to be the result of inhibition of the reaction system in the Calvin-Benson Cycle, in which sugar is synthesize from corbondioxide, by magnetic field with higher magnetic flux densities. That is the inhibition of sugar synthesis by the existence of a magnetic field is thought to be one of the cause for the growth inhibition of *S. maxima* under magnetic flux densities of 400 G. Takahashi *et al* investigated the relation between the growth of green algae *chlorella* and the magnetic flux density and supposed that the light excitation is considered to be closely related to the easy formation of chlorophyll redical pairs in the presence of magnetic field and to some influence of magnetic field on the concentration of photosynthetic pigments like phycocyanin antenna cholophyll and β carotene which supply light energy to the reaction center cholophyll in the thylakoid membrane.

The study focused on the therapeutically potential of the *S. maxima* in experimentally induced diabetic animals. The experimental diabetes induced by fructose diet eventually caused the hyperglycemic state after 6 month duration. Similar results were also observed with Yoshino (1992). It was also reported that genetically modified rats developed diabetes and diabetic angiopathy when fed with 72% fructose diet for 2 months, but not

when fed a 72% cornstarch diet. Hence in the present study the blood glucose level elevations were attained only after 6 months. Similar study made on the with 10 %, 20 % fructose administration orally elevated the increases in blood pressure within a week and followed by disturbances in the metabolism of glucose and triglycerides (Dai and Mcneill 1995). It was also reported by Cohen *et al* (1977) that long-term fructose feeding studies (80% sugar diets for 26 weeks) have been conducted in rats resulted in insulin resistance. This method of developing insulin resistance induced hyperglycemia mimics the mechanism of occurrence of type -II diabetes in humans.

The present study is divided into two phases the animals kept on overnight fasting as well as animals kept on non fasting state. Increased hepatic gluconeogenesis is a predominant cause of postprandial hyperglycemia in type 2 diabetes, fructose 1,6-bisphphosphatase (FBPase) is the key enzyme elevated during the fasting condition as well as in post prandial condition. The present study is focused on the blood glucose reduction efficacy of protein dosage of *S. maxima* administered in diabetic or normal animals either at overnight fasted state or non fasted state.

It was observed from the present study that animals fed with fructose rich diet had a fasting blood glucose levels (FBG) ranging between 100mg/dl – 125 mg/dl. Whereas the normal animals had a FBG levels ranging between 70mg/dl – 90 mg/dl after overnight fasting, these results are in accordance with fasting plasma glucose levels of rats with mild diabetes that is 7.4 +/- 0.9 (mean +/- SD) (mmol/l) (133mg/dl), whereas 6.5 +/- 0.6, (mean +/- SD) (mmol/l) (117 mg/dl) was observed with normal rats pugiliese *et al*., (1989).

It was observed from the study that EMF unexposed *Spirulina maxima* administration in normal animals showed the highest reduction in blood glucose levels and this reduction was found to be insignificant with EMF exposed as well as diabetic control, the results concludes that the administration of *S. maxima* doesn't lead to hypoglycemic condition, Layam *et al*., (2006) revealed that administration of *Spirulina* reverse the diabetic condition, and this effect is due to the potentiation of the pancreatic secretion of insulin from islet β-cell or due to enhanced transport of blood glucose to the peripheral tissue. When O/N fasted mild diabetic animals were treated with different exposures of *Spirulina* dosages, It was observed that the diabetic animals showed a significant decrease in the case of EMF unexposed *Spirulina* treated group. However subsequent

reduction in the control group was also observed in the case of overnight fasted animals hence the same dosage of *Spirulina* was administered in non fasted animals. Incase of the non-fasted mild diabetic animals administered with 500 mg of the protein dosage of *S. maxima*. It was observed from the study that blood glucose levels were found to be reduced in the case of EMF unexposed and EMF 400 G exposed *S. maxima* as compared with the control. Similar effect was observed by Davidson 1981, coined the auto regulation property of glucose modulating the glycogen and glycolytic pathways but did not affect gluconeogenesis. It was observed from the study of Davidson diabetic rats lacked autoregulation of leading to curtailing of glycolysis and increase in the rate of gluconeogensis. The present study 72 % of fructose diet for 6 months had deregulated the glucose homeostasis moderately and thereby leading to the mild diabetes. It was observed from the study that non-fasted normal animals showed a significant reduction in the blood glucose in case of EMF un exposed as well as EMF exposed (400G). From the overall study it could be concluded that the exposure of EMF to *S. maxima* alleviates only its biomolecular constituents whereas the antidiabetic property remained unaffected.

CONCLUSION

The cultivation of *S.maxima* under Electromagnetic field in airlift photobioreactor, under various intensities of magnetic field ranging from 1 G- 400 G, it was concluded from the study that magnetic field of 1G intensity enhances the growth rate, chlorophyll rate whereas EMF intensity of 400 G inhibit the growth of *S. maxima* at a greater extent. The *in vivo* studies revealed a significant reduction in the blood glucose levels in EMF control or EMF exposed. The M.S was developed in rats after 6 months of high fructose feeding thereby leading to mild diabetic condition. Screening of antidiabetic potential of *S. maxima* (either EMF exposed or unexposed) in experimental diabetic rats showed blood glucose reduction which was found to be significant. The insulin assay revealed that insulin levels were found to be significantly increased after the *S.maxima* administration.

Summary

The overall study reveals that EMF exposure enhances the growth of *S. maxima* Whereas the higher intensities of EMF retards the growth rate and thereby extends the generation time of *S. maxima*. The primary metabolite content was also found to decline at high EMF intensities(400G). It was evident when the exposed and the control *S. maxima* were subjected to the IR spectroscopy , a decrease in the levels of NH group or lack of strong bands for –NH in the EMF exposed *S.maxima*. The Electron microscopic studies revealed a distinct septum formation and loosening of the L-II & L-III layers of the cell/ trichome, that feature was only observed with the EMF 1G exposed spirulina., which is one of the characteristic feature of cell division. Moreover the Isoleucine residues which are only found in 1G EMF exposed *S.maxima* is another indication of the influence of EMF on the cell division.

It was observed from the study that both EMF exposed and control showed the blood glucose lowering activity and that was observed at either fasting condition or nonfasted conditions. The insulin assay revealed that insulin levels were found to be significantly increased after the *S.maxima* administration. Hence it was understood that the EMF exposure hasn't influenced any change in the active principle.

It was also observed from the study medium optimization is totally dependent on the metabolite or active principle of Spirulina. Since it was observed from the study that the primary m,etabolite content is elevated only when cultivated in zarrouks medium whereas the secondary metabolites content was elevated when cultured in the modified zarrouks medium. Moreover the rate of production of the primary metabolites mimicks the first order kinetics reaction. Further studies are required to identify the active principle and thereby enhance the scale up production of the metabolite with the optimizations attained in the study.

The overall study concludes that cultivation of *S.maxima* under 1G EMF can enhance the biomass.

CHAPTER: 3 :

Additional Studies Undertaken (other than proposed objectives)

Medium optimization: 1

Biomass production of S. *maxima* by modulating the compositions of Nutrient Supplementation

Introduction

Spirulina is a microscopic and filamentous cyanobacterium that belongs to family Oscillatoriaceae and has a long history of use as food and food supplement. It is now being widely studied for its possible antioxidant (Estrada et al., 2001) antiviral (Kulshrestha et al.,2008), anticancer (Khan et al., 2005), antibacterial, and antiparasitic properties, and has been used for such medical conditions as allergies, ulcers, anemia, heavy-metal poisoning, and radiation poisoning. On a dry-weight basis, the cyanobacterium Spirulina is composed of 50–70% proteins, 5–10% lipids and 10–20% carbohydrates (Vonshak et al., 1997) and possesses an elevated content of compounds of high nutritional value such as provitamins, minerals, polyunsaturated fatty acids such as gamma-linolenic acid (Miranda et al., 1998) and phycocyanin (Tanticharoen et al., 1994; Cohen et al., 1993). Modifications in the medium composition may transmit in changes in growth rate, biochemical and neutraceutical composition of *S.maxima*

ere made up to enhance the biomass rate, hence *Spirulina maxima* was cultured in different growth medium and productivity in terms of growth rate, levels of primary and secondary metabolites. The variations in the microelements doesn't show any observable difference in biomass and metabolite rate. Large-scale production of *Spirulina maxima* is hampered by problems associated with process design. Low productivity with typical cell

densities of 0.5– 1 g L^{-1} is a major obstacle to the successful commercialization of *Spirulina maxima* (Richmond .(1992), The optimization and control of bioprocesses often requires the establishment of a mathematical model that describes the kinetics of process variables (microbial growth, substrate uptake and product formation). Despite impressive progress made recently in developing structured models for microbial growth, the unstructured models or semi-mechanistic models are still the most popular ones used in practice (Zeng et al., 1995). The present study is focused on the designing a proper medium for the cultivation of *S.maxima.*

The data was analysed statistically by one-way analysis of variance (ANOVA) using Sigma STAT version 2.0 and principal factor component analysis (unrotated) was performed using manitab 15. The principal component analysis was performed to analyse the largest data variation among the fewest number of components. The principal component factor score was also represented in the form of eigen values, which are non zero vectors of the eigene vectors. The maximum eigen values correspond to maximum influence observed with the experimental data.

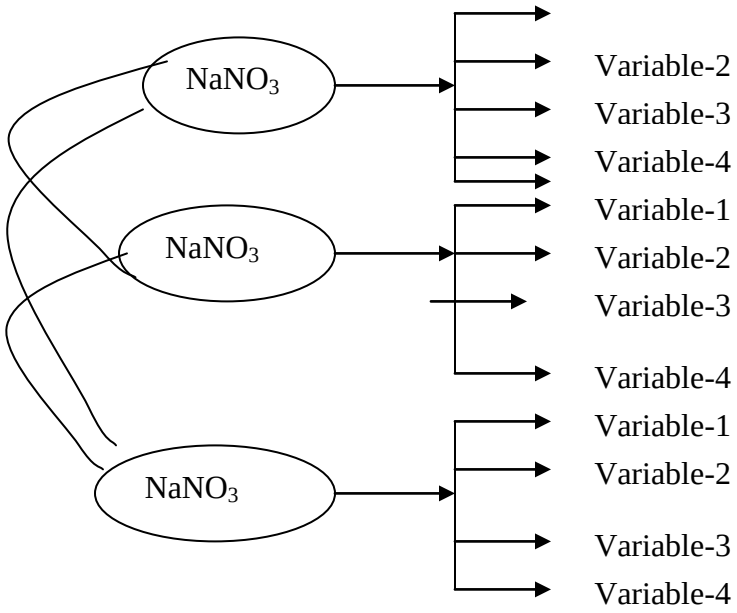


Fig31: Schematic diagram to show the Factor analysis

The impact of diverse nitrogen sources on the growth and neutraceutical composition of *Spirulina* had earlier been studied by various workers and the best results for the biomass production were attributed to the use of nitrates (Faintuch 1989; Colla et al. 2007). The metabolites of *S. maxima* was monitored for 28 days however the maximum metabolite yield was monitored only after 21 days and the levels tend to decline hence the observations were made upto three weeks. It was the study that, increasing concentrations of sodium nitrate influences the growth rate and metabolites of *S. maxima*.

Such as chlorophyll-a , carbohydrate and ascorbic acid Table (4) whereas the lower concentration also influences the protein content, lipid content and phenolocs as well. The factor scores of the variables revealed the positive and the negative influence of Sodium nitrate concentrations Table (5).

Maximum growth μ (0.00424 μ/h) and biomass (3.18g/l) were found at 3.0g/l sodium nitrate concentration (**Fig2**). An increment of 11.18% and 4.41% in *S. maxima* biomass and growth rate respectively were observed in comparison to the *S. maxima* grown in control medium (Zarrouk's medium with 2.5g/l). The sodium nitrate not only influenced the growth and biomass of *S. maxima* but also affected the biochemical composition such as protein, lipid, carbohydrate, phenol and ascorbic acid. A significant increase ($P<0.001$) of 6.74 % in protein was obtained at 3.0 g/l concentration of sodium nitrate (Fig34).

The principal component analysis revealed that protein composition was negatively coorelated at high concentrations of Sodium nitrate, Moreover some intermediate

positive coorelations were also observed with proteins at some sodium nitrate concentrations.

Nitrogen is required for synthesis of the amino acids, which make up protein and other cellular components. Similarly a significant increase ($P<0.001$) in the lipid content (7.47%) at 3g/l concentration of sodium nitrate was recorded (**Fig-32**). Manabe et al. (1992) also reported higher total lipid content in *Spirulina* grown in medium containing 25mM of ammonium chloride as nitrogen source. It was reported that total lipid content significantly increased at 2.5g/l of sodium nitrate concentration in Zarrouk's medium (Colla et al. 2007). Carbohydrate content of the *S. maxima* gradually increased with increasing $NaNO_3$ as expected since the carbohydrate content of the cell is directly proportional to biomass content (**Fig34**). Higher concentrations of sodium nitrate promoted the phenolic content of *S. maxima* (**Fig-33**). The mechanism(s) used by cyanobacterium to synthesize phenolics and their metabolic functions remain obscure (Colla et al. 2007). The sodium nitrate content in the medium also influenced the pigment composition of *S. maxima* significantly. Chlorophyll-*a* is the major photosynthetic pigment found in *S. maxima.* Chlorophyll was found to be positively coorelated with increasing sodium nitrate concentrations. A significant increase ($P<0.001$) was observed in chlorophyll-*a* (15.61%), phycocyanin (12.76%), phycoerythrin (5.85%) and allophycocyanin (7.52 %) at 3.0g/l concentration of sodium nitrate (**Table 6**). It was observed that the accessory pigments such as allophycocyanin, phycoerythrin, phycocyanin was positively coorelated only at the lower concentration of NaNo3 and the optimum was 3.0 as observed with the experimental evidence.A significant increase ($P<0.001$) (11.24 %) in carotenoid content (**Fig-33**) was also observed at 3.0g/l concentration of sodium nitrate. The importance of the nitrogen

source for the integrity of photosynthetic apparatus was well established (DeLoura et al. 1986; Allen and Smith 1969). A significant increase ($P<0.001$) (17.59%) in ascorbic acid content was observed at 4.5g/l concentration of sodium nitrate (**Fig-33**). Despite the fact that phycocyanin and phycoerythrin content was high at higher concentration of sodium nitrate(4.0 gl^{-1}), the biomass and other neutraceuticals such as proteins, lipids, phenols, chlorophyll-*a*, carotenoids, and allophycocyanin were at their maximum at 3.0g/l of sodium nitrate. The natural preparations rich in neutraceuticals help in preventing the diseases or lessening the severity of the diseases (Estrada et al. 2001; Hu et al. 2000) and *Spirulina* is best known for therapeutic value and cultivation of the cyanobacterium at optimum concentration of nitrogen source enriches the neutraceutical composition of the organism.

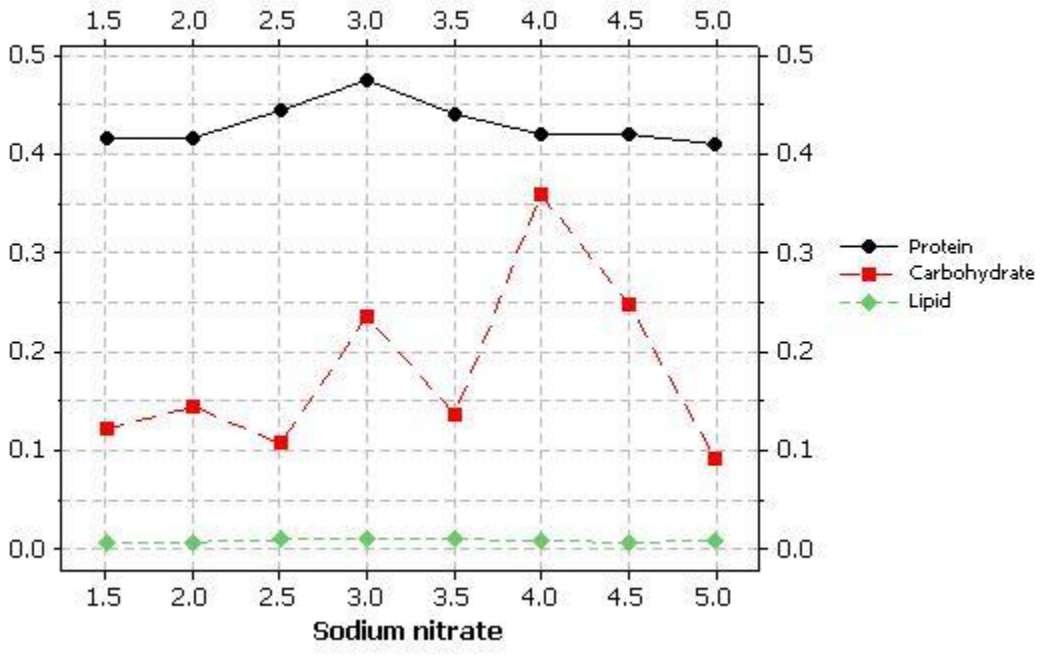


Fig -32 : Protein, carbohydrate and lipid content of *S.maxima* cultured under different sodium nitrate concentrations.

The init of protein expressed as mg/ml

The unit of carbohydrate expressed as mg/ml

The unit of lipid expressed as mg/l

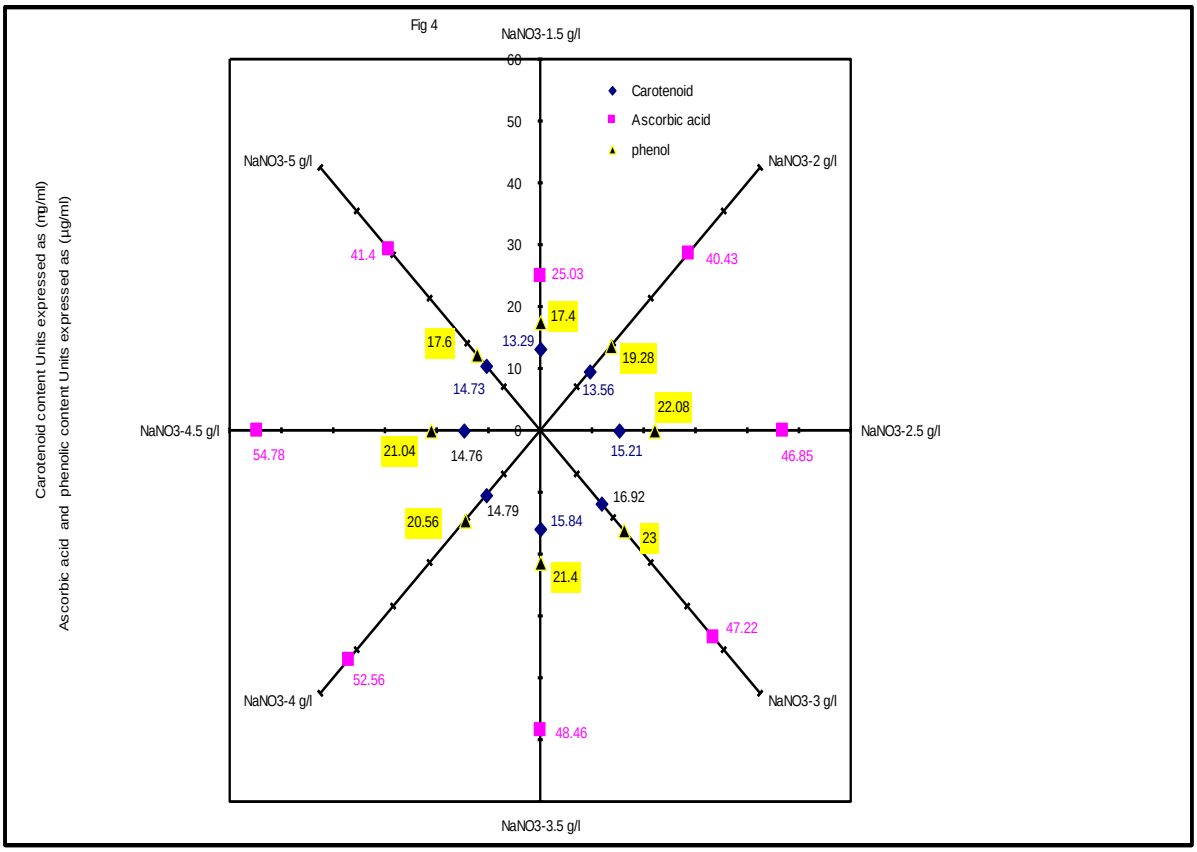


Fig -33 : Non enzymatic antioxidants of *Spirulina maxima* cultured under different sodium nitrate concentrations.

Unit of carotenoid expressed as mg/ml

Unit of Ascorbic acid expressed as µg/ml

Unit of phenol expressed as μg/ml

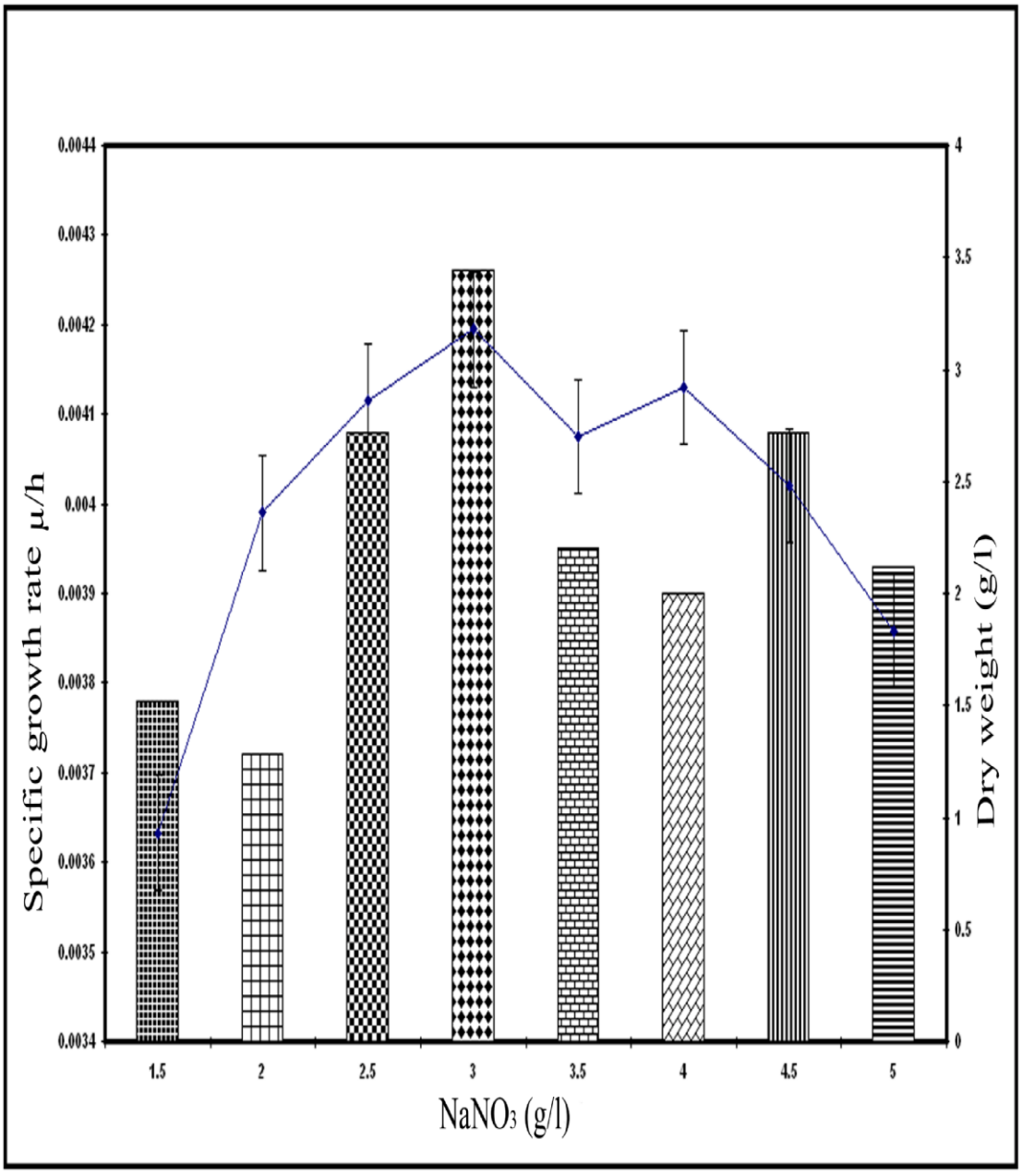


Fig -34 : Biomass and specific growth rate of *Spirulina maxima* cultured under different sodium nitrate concentrations.

Unit of Biomass expressed as g/l

Unit of Specific growth rate is μ/h

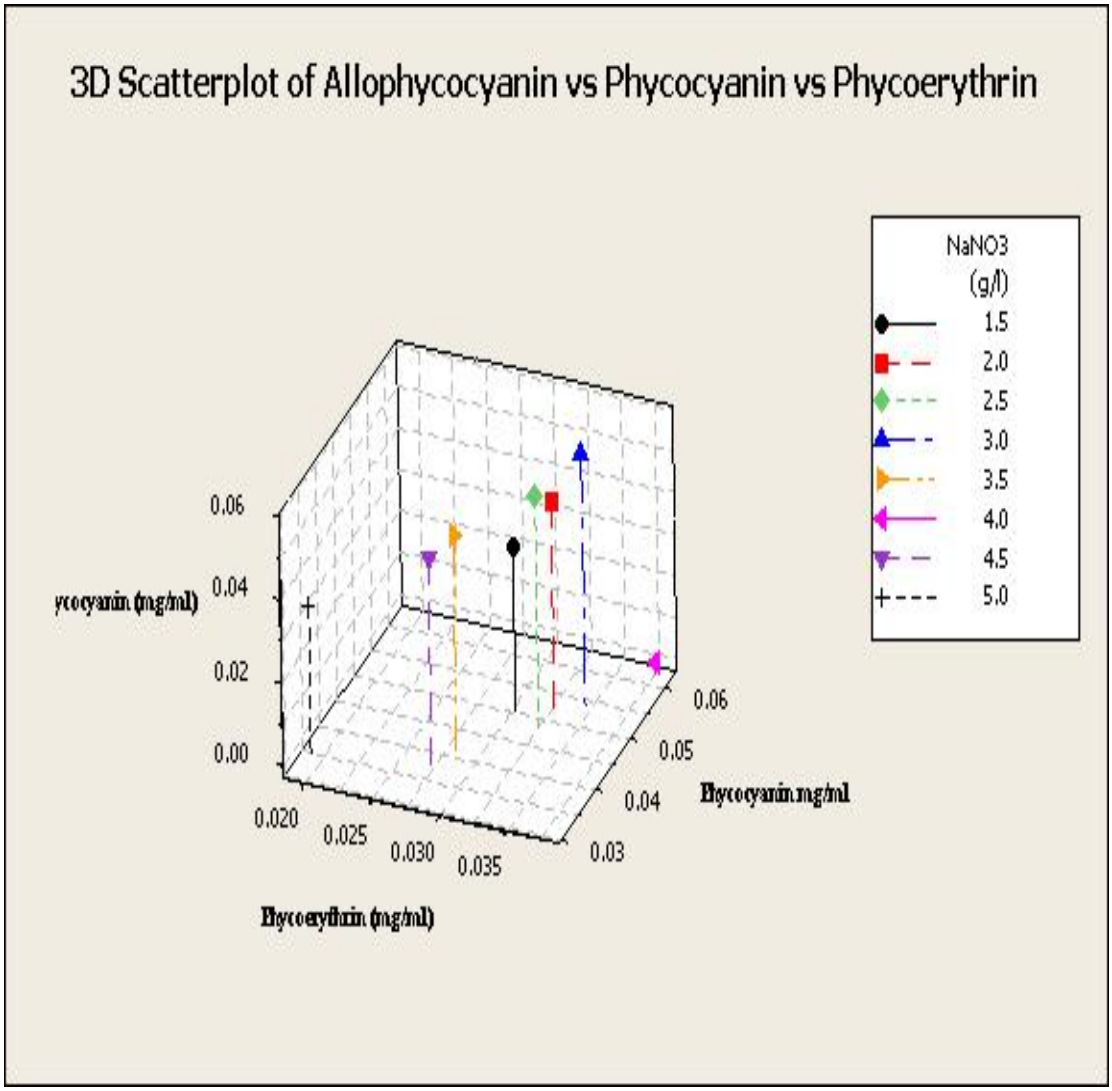


Fig -35 : Phycobiliprotein content of *Spirulina maxima* cultured under different sodium nitrate concentrations.

Unit of Phycocyanin expressed as mg/ml

Unit of Allo phycocyanin expressed as mg/ml

Unit of Phycoerythrin expressed as mg/ml

Eigenanalysis of the Correlation Matrix								
Eigenvalue	4.5572	1.6339	0.9437	0.5041	0.2658	0.0915	0.0037	-0.0000
Proportion	0.570	0.204	0.118	0.063	0.033	0.011	0.000	-0.000
Cumulative	0.570	0.774	0.892	0.955	0.988	1.000	1.000	1.000
Variable		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Sodium nitrate		0.200	0.582	0.491	-0.198	0.186	-0.280	-0.262	-0.399
Chlorophyll		0.435	0.073	0.129	0.052	0.493	0.721	0.087	0.125
Protein		0.364	-0.448	-0.181	-0.105	0.303	-0.292	0.383	-0.547
Carbohydrates		0.225	0.417	-0.522	0.659	0.134	-0.217	-0.057	0.004
Lipids		0.301	-0.262	0.512	0.581	-0.465	0.053	0.007	-0.148
Carotenoids		0.442	-0.175	0.164	-0.111	0.152	-0.482	-0.083	0.687
Ascorbic acid		0.364	0.395	-0.137	-0.305	-0.530	0.068	0.554	0.082
Phenols		0.411	-0.162	-0.359	-0.262	-0.306	0.169	-0.678	-0.159

Table- 5: Principal Component Analysis: of S. maxima cultured in different concentrations of Sodium nitrate

Table-6(a)

Chlorophyll-*a*, carotenoid and phycobiliprotein contents of *S. maxima* grown in Zarrouk's medium containing different concentrations of sodium nitrate

S.No	Sodium Nitrate (mg /ml)	Chlorophyll-*a* (mg/ml)	Carotenoid (mg/ml)	Phycobiliproteins (mg/ml)		
				Phycocyanin	Allophycocyanin	Phycoerythrin
1	1.5	55.29± 0.317 **	13.29 ± 0.001 **	0.05 ± 0.0007 **	0.036 ± 0.0002 **	0.03 ± 0.0002
2	2.0	54.83 ± 0.446 **	13.56 ± 0.003 **	0.052 ± 0.0001 **	0.047 ± 0.0001 **	0.032 ± 0.0002
3	2.5	76.09 ± 0.379 **	15.21 ± 0.005 **	0.047 ± 0.0004 **	0.053 ± 0.0001 **	0.032 ± 0.0004
4	3.0	87.97 ± 0.317 ** 15.61%	16.92 ± 0.003 ** 11.24%	0.053 ± 0.0003 ** 12.76 %	0.057 ± 0.0003 ** 5.85%	0.034 ± 0.0002 7.523 %
5	3.5	68.16 ± 0.189	15.84 ± 0.003 **	0.040 ± 0.0003 **	0.049 ± 0.0003 **	0.028 ± 0.0002
6	4.0	76.50 ± 0.087	14.79 ± 0.012 **	0.061 ± 0.0001 **	0.027 ± 0.0001 **	0.037 ± 0.0005
7	4.5	74.62 ± 0.37 9 **	14.76 ± 0.001 **	0.036 ± 0.0003 **	0.048 ± 0.0003 **	0.027 ± 0.0009
8	5.0	72.64 ± 0.577 **	14.73 ± 0.001 **	0.033 ± 0.00001 **	0.034 ± 0.0001 **	0.019 ± 0.0001

Values are expressed in mean ± Standard Error

Percentage increase was calculated in comparison to the sodium nitrate concentration of standard Zarrouk's medium.

p<0.05 (*), p<0.001 (**) significant in comparison to the sodium nitrate concentration of standard Zarrouk's medium.

Table: 6(b)

Protein, carbohydrate, lipid, Ascorbic acid, phenol contents of *S. maxima* grown in Zarrouk's medium containing different concentrations of sodium nitrate

$NaNO_3$ Conc.	Protein	Carbohydrate	Lipid	Ascorbic acid	Phenol
1.5	0.4178±0.005 (**)	122.5±0.00177(**)	7.42±0.296985(**)	25.03945±0.11882(**)	17.4 ±0.001768(**)
2.0	0.4178±0.001(**)	144.17±0.00177(**)	7.28±0.39598(**)	40.43283±0.02376(**)	19.28 ±0.000707 (**)
2.5	0.4459±0.003	107.5±0.00318	9.66±0.098995	46.58346±0.04753	22.08±0.000707
3.0	0.4753±0.00176(**)	235.17±0.00707(**)	10.36±0.09799(**)	47.22205±0.11882	23±0.003536
3.5	0.4459±0.00353	137.4177±0.00354(**)	10.08±0.05799(**)	48.46562±0.04753(**)	21.4±0.001768 (**)
4.0	0.4211±0.007(**)	360.07±0.00106(**)	9.8±0.06799	52.56604±0.09506(**)	20.56±0.00707
4.5	0.4244±0.001(**)	248.74±0.00460(**)	6.291993±0.19799(**)	54.7843±0.23765(**)	21.04±0.003536
5.0	0.4112±0.005(**)	91.9204±0.00247(**)	9.465±0.159099	41.40752±11.9779(**)	17.6±0.002121 (**)

Values are expressed in mean ± Standard Error

Percentage increase was calculated in comparison to the sodium nitrate concentration of standard Zarrouk's medium.

p<0.05 (*), p<0.001 (**) significant in comparison to the sodium nitrate concentration of standard Zarrouk's medium.

SUMMARY OF THE RESULTS (In nutshell)

Original objectives proposed	Objectives Achieved
Evalation of efffect of EMF on following parameters :	
1. Spirulina Biomass production	**EMF exposure enhances the biomass production**
2. Nutritional Composition	**Elevates protein content when exposed to combined effect of 1G EMF and high light intensities.**
3. Chlorophyll-a content	**Elevates chlorophyll-a content when exposed to 1G EMF with 5000 lux intensity**
4. Antioxidants such as phycocyanin, carotenoid levels	**Elevates carotenoid content when exposed to 1G EMF (irrespective of light intensity)**
5. Andiabetic activity	**EMF 400 G exposed (Higher intensity) and EMF control showed blood glucose lowering effect (biological activity of *S.maxima* remained unchanged even after 400 G EMF exposure)**

Bibliography

Allen MM, Smith AJ (1969) Nitrogen chlorosis in blue-green algae. Arch Microbiol. 69: 114-120

Ayehunie S., Belay A., Baba T.W., Ruprecht R.M. (1998).“Inhibition of HIV-1, replication by an aqueous Extract of Spirulina platensis (Arthospira platensis).” JAIDS: Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology. 18: 7-12.

Basciano H, Federico L, Adeli K (2005) Fructose, insulin resistance, and metabolic dyslipidemia. Nutr & Metab. 2: 01-14.

Bates DM and Watts DG (1988) Nonlinear Regression Analysis & its applications.

Baynes JW, Thrope SR. (1996) The role of oxidative stress in development of complications in diabetes. Curr Opinion Endo. 3: 277-284.

Becker E.W (1984). Nutritional properties of microalgal potentials and constraints. In: Richmond A, Ed. Handbook of microalgal mass culture. CRC Press, Inc, Boca Ratón; pp. 339-408.

Becker EW, Jakober B, Luft D, Schmuling RM. (1986) Clinical and biochemical evaluations of the algae Spirulina with regards to its application in treatment of obesity: A double blind cross over study. Nutr reports Internat’l. 33: 565-574.

Belay A., (1993). Current knowledge on potential health benefits of Spirulina. Journal Applied Phycology. 5: 235-241.

Belay A., (1997). Mass culture of Spirulina outdoors. –The Earthrise Farms experience. In: Vonshak, A., Ed. Spirulina platensis (Arthrospira): Physiology, cell-biology and biotechnology. Taylor and Francis. London. pp. 131-158.

Belay, A. (1997) Physiology, Cell-Biology and biotechnology Taylor and Francis, London, pp. 131-158.

Belay, A.; Kato, T. and Ota, Y. (1996) J. Appl. Phycol. 8: 303-311.

Belay, A.; Ota, Y.; Miyakawa, K. and Shimamatsu, H. (1994) Algal Biotechnology in the Asia-Pacific Region, University of Malaya. 92-102.

Bennet A, BogoradbL (1973) Complementary chromatic adaptation in a filamentous blue green alga. J. cell Biol. 58: 419- 435.

.

Bhadauriya P, Gupta R, Singh S, Bisen PS (2007). Physiological and Biochemical alterations in diazotrophic cyanobacterium Anabaena cylindrica under NaCl stress. Curr Microbiol 55: 334-338.

Bhadouriya P, Gupta R, Singh S, Bisen PS, (2008). n-Alkanes variability in the diazotrophic cyanobacterium anabena cylindrical in response to Nacl stress. 24: 139-141.

Bharti S, Garg OP (1970). Effect of varing number of short days, gibberelic acid and ascorbic acid on the flowering of soybean. Plant Cell Phisiol 11: 657-662.

Bharti S, Garg OP, (1970). Effect of varying no of short days , gibberelic acid and ascorbic acid on the flowering of soya bean. Plant and cell physiology. 11 (4),657-662.

Blackman C.F., Benane S.G., Ellioit D.J., Pollock M.M.,(1988). Influence of electromagnetic fields on the efflux of calcium ions from brain tissue in vitro: a three-model analysis consistent with frequency response to 510 Hz. Bioelectromagnetics. 9: 215-227.

Busson F., (1971). Spirulina platensis (Gom.) Geitler et Spirulina geitleri J.de Toni, Cyanphycees Alimentaires, Service de Sante, Marseille.

Caller M, Mackinney G (1965). The carotenoids of certain fruits (apple, pear, cherry, strawberry). J Food Sci 30: 393-395.

Calorimetric method for determination of sugar and related substances. Analytical chemistry. 300-356.

Chaumont D., (1993). Biotechnology of algal biomass production; review of systems for outdoor mass culture, J. Appl. Phycol. 5:593-604.

Chen F, Johns MR (1994). Substrate inhibition of Chlamydomonas reinhardtii by acetate in heterotrophic culture. Process Biochem.. 29: 245–252.

Ciferri O (1983). Spirulina, the edible microorganism. Microbiol. Rev 47: 551-57.

Clement G (1971) une nouvelle algue alimentaire: la Spirulina, rev. Inst. Pasteur, 4, 103.
Cohen A. M., Teitelbaum A., Rosenmann E (1977). Diabetes induced by a high fructose diet. Metabolism. 26: 17-25.

Cohen Z., (1997). The chemicals of Spirulina. In: Vonshak, A., Ed. Spirulina platensis (Arthrospira): Physiology, cell-biology and biotechnology. Taylor and Francis. London. pp. 175 – 204.

Colla LM, Furlong EB, Costa JAV (2007). Brazilian archives of Biology and Technology 50: 161- 167.
Davidson MB (1981). Autoregulation by glucose of hepatic glucose balance: permissive effect of insulin. Metabolism. 30(3): 279-84.

Day JG, Tsavalos AJ (1996) An investigation of the heterotrophic culture of the green alga Tetraselmis. J. appl. Phycol. 8: 73–77.

De Caire G.Z., De Cano M. S., De Mule M. Z., Steyerthal N., Piantanida M (1995). Effect of Spirulina plarensis on glucose, uric acid and cholesterol levels in the blood of rodents. International Journal of Experimental Botany. 57: 93-96.

DeLoura IC, Dubacq JP, Thomas JC (1986). The effects of Nitrogen deficiency on pigments and lipids of cyanobacteria. Plant physiol 83: 838– 843.

Dillon J.C., and Phan P.A.(1993). Spirulina as a source of proteins in human nutrition. In: Doumengue, F., Durand-Chastel, H., Toulemont A, Eds. Spiruline algue de vie. Musée Océanographique. Bulletin de l´Institut Océanographique Monaco. Numéro spécial 12:103-107.

Dubacq J.P., Pham-quoc K., (1993). Biotechnology of Spirulina lipids: a source of gamma-linolenic acid. In: Doumengue, F., Durand-Chastel, H., Toulemont, A., Eds. Spiruline algue de vie. Musée Océanographique. Bulletin de l´Institut Océanographique Monaco. Numéro spécial 12:59-64.

Dubois M, Gilles KA, Hamilton JK et al (1956). Calorimetric method for determination of sugar and related substances. Anal Chem 28: 300- 356

Estrada JE, Bescos P, Del Frensno AMV (2001). Antioxidant activity of different fractions of Spirulina platensis protean extract. II Farmaco 56: 497- 500.

Estrada JE, Bescos P, Villar Del Frensno AM (2001). Antioxidant activity of different fractions of Spirulina platensis protean extract .II Farmaco. 56: 497-500.

Faintuch BL., Sato S., Aquarone E., (1991). Influence of the nutritional sources on the growth rate of cyanobacteria. Archives of Biological Technologies. 34: 13-30.

Faure P, Rossini E, Lafond JL, Richard MJ, Favier A, Halimi S. Vitamin E improves the free radical defense system potential and insulin sensitivity of rat fed high fructose diets. J Nutr. 1997; (127): 103-107.

Folch J, Lees M (1957). A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509.

Fox D (1993). Health benefits of Spirulina and proposal for a nutrition test on children suffering from kwashiorkor and marasmus. In: Doumengue, F., Durand-Chastel, H., Toulemont, A., Eds. Spiruline algue de vie. Bulletin de l´Institut Océanographique Monaco, Musée Océanographique. Numéro spécial 12: 179-185.

Fox R. (1998). Nutrient preparation and low cost basin construction for village production of Spirulina In: Stadler, T., Mollion, J., Verdus, M.C., Karamanos, Y., Morvan, H., Christiasen, D., Eds. Algal Biotechnology. Proceedings of the 4th International Meeting of the SAA. Elsevier Applied Science, London – New York; pp. 355-364.

Fronzo De, Bonadonna RA, and Ferrannini E. (1997) Pathogenesis of NIIDM, Internatinal Text Book of Diabetes Mellitus, 2nd ed. Chichester, John Wiley, England. pp. 365-712.
Gen Yoshino (1992). Effect of mild diabetes and dietary fructose on very-low-density lipoprotein triglyceride turnover in rats. Metabolism. 41(3): 236-240.

Genkov D., Cyetkova A., and Atmadzov P (1974). The effect of the constant magnetic field upon the growth and development of Tr. Vaginalis. Folina Medica, 16: 95-99.
Gonzalez De Rivera C, Miranda-Zamora R, Pardes-Carbajal MC, Juarez Oropeza, MA (1993). Preventive effects of Spirulina maxima on the fatty liver induced by a fructose rich diet in the rat, a preliminary report. Life Sc.53(7): 57-61.

Gonzalez R., Romay C., Ledon N (1999). Phycocyanin extract reduces leukotriene B4 levels in arachidonic acid-induced mouse-ear inflammation test. J pharm. Pharmacol. 51: 641-642.

Goodman E.M., Greenebaum B., & Marron M.T., (1994). Magnetic fields after translation in Escherichia coli. Bioelectromagnetics, 15: 77-83.

Gorban, E. M., Orynchak, M. A., Virstiuk N. G., Kuprash L. P., Panteleimonov T. M., Sharabura L.B., (2000). Clinical and experimental study of Spirulina efficacy in chronic diffuse liver diseases. Lik Sprava. 6: 89-93.

Gupta R, Bhadauriya P, Chauhan VS et al (2008) Impact of U-V –B radiation on thylakoid membrane and fatty acid profile of Spirulina platensis. Curr Microbiol 56: 156 – 161

Gupta R, Bhadauriya P, Chauhan VS, Bisen PS, 2008. Impact of UV- B radiation on thylakoid membrane and fattynacid profile of Spirulina platensis. Current microbiology 56, 156-161.

Hayashi K., (1996)a. Calcium-Spirulan, an inhibitor of enveloped virus replication, from a blue-green alga Spirulina. J Nat Prod. 59:83-87.

Hayashi K., Hayashi T., and Kojima I., (1996)b. A natural sulfated polysaccharide, Calcium-Spirulan, isolated from Spirulina platensis: in vitro and ex vivo evaluation of anti-herpes simples virus and anti-human immunodeficiency virus activities. AIDS Res. Hum. Retroviruses. 12:1463-1471.

Hirahashi T., Matsumoto M., Hazeki K., Saeki Y., UI M., Seya T (2002). Activation of the human innate immune system by Spirulina augmentation of interferon production and NK cytotoxicity by oral administration of hot water of Spirulina platensis. International Immunopharmacology. 2: 423-34.

Hosoyamada Y., Takai T., Kato T (1991). Effects of water-soluble and insoluble fractions of Spirulina on serum lipid components and glucose tolerance in rats. Journal of japan Soiety Nutrition Food Science. 44: 273-277.

Hu FB, Rimm EB, Stampfer MJ, Ascherio A et al (2000). Prospective study of major dietary patterns and risk of coronary heart disease in men. American Journal of Clinical Nutrition 72: 912–921.

Ishii K., Katoch T., Okuwaki Y., Hayashi O (1999). Influence of dietary Spirulina platensis on Iga level in human saliva. J Kagawa Nutr Univ. 30: 27-33.

Itawa K, Inayama T (1990). Effect of Spirulina platensis on plasma lipoprotein lipase activity in fructose induced hyperlipidemic rats. J Nutr Sci Vitaminol. 36(2):165- 171.

Johns MR, Tan CK and Chen F (1989). Heterotrophic microalgal production of chemicals. In: Proceedings of the Eighth Australian, Biotechnology Conference, University of New South Wales, Sydney, pp 542–545.

Kato T., Takemoto K., Katayama H., Kuwabara Y., (1984). Effects of Spirulina (Spirulina platensis) on dietary hypercholesterolemia in rats. Journal of Japan Society Nutrition Food Science. 37: 323-332.

Kato, T.; Takemoto, K.; Katayama, H. and Kuwabara, Y. (1984). J.Jap. Soc. Nutr. Food Sci., 37: 323-332.

Khan Z, Bhadauriya P, Bisen PS (2005). Nutritional and therapeutic potential of Spirulina. Curr Pharma Biotech 6: 373- 379.

Kimball, G.K., (1938).The growth of yeast in a magnetic field. J. Bact., 35, 109-122.

King H., Albert, RE., Herman, WH. (1998). Global burden of diabetes, 1995 – 2025: prevalence numerical estimates, and projection. Diabetes Care 21 (9): 1414-31.

King H., Aubert R.E., Herman W.H. (1998). Type II diabetes mellitus. Diab. Care, 21: 1414-1431.

Koji Tsuchiya., Kazuhira N., Kazumasa O., Takashi A., and Makoto S (1996). Effect of homogeneous and inhomogeneous high magnetic field on the growth of Escherichia coli. Journal of Fermentation And Bioengineering. 81,4: 343-346.

Kratz WA, Meyers J (1955). Nutrition and growth of several Blue green algae. Am Journal Bot 42: 282- 287.

Kulshreshtha A, Zacharia JA, Jarouliya U, Bhadauriya P, Prasad GBKS, Bisen PS (2008). Spirulina in health care management. Current pharmaceutical Biotechnology. 9 (5) 400- 405.
L.V.Venkataraman (1983) , A monograph , published by CFTRI Mysore , India.

Layam A., Reddy C. L. K (2006). Antidiabetic property of Spirulina. Diabetologia Croatica; 35-2.
Lee Y. K (2001). Microalgal mass culture systems and meyhods: Their limitations and potential. Journal of Applied Phycology. 13: 307-315.

Lowry H, Rosenburg NJ, Farr AL et al (1951). Protein measuring with Folin phenol reagent. J Biolo Chem. 193: 265-275.

Mackinney G (1941). Absorption of light by chlorophyll solutions. Journal of biological chemistry. 315 -321.

Manbe E, Hirano M, Takano H et al (1992). Influence of ammonium chloride on growth and fatty acid production by Spirulina platensis. Appl Biochem Biotechnol 35: 273– 281.

Mani S., Iyer U., Subramanian S., (1998). Studies on the effect of Spirulina supplementation in control of diabetes mellitus In: Subramanian G, eds Cyanobacterial Biotechnology. USA: Science Publishers. 301-304.

Manoj, G.; Venkataraman, L.V. and Srinivas, L. (1992) In ETTA National Symposium on Spirulina, (Sheshadri, C.V.; Jeejibai, N.; Eds.), MCRC Publishers, pp. 148-154.

Miranda MS, Cintra RG, Barros SBM (1998). Antioxidant activity of microalga Spirulina maxima. Braz J Med Biol Res 31: 1075- 1079 .

Moore R. L (1979).. Biological effects of magnetic fields: studies with microganisms. Can. J. Microbiol. 25: 1145-1151.

Moore R., Barchi J., and Patterson G (1984). Acutiphicin and 20,21-didehydroacutiphicin, new antineoplasic agents from the cyanophyta Oscillatoria acutissima. J Am Chem. Soc 106: 8193-8197.

Moore R., Mynderse J., Kashiwagi M., and Norton T (1977). Antileukemia activity in the oscillatoriaceae: isolation of debromoaplysiatoxin from Lyngbya. Science 196:538-539.

Morio Hirano., Akira Ohta and Katsuya Abe (1998). Magnetic field effects on photosynthesis and growth of the cyanobacterium Spirulina platensis. Journal of Fermentation and Bioengineering. 86(3): 313-316.

Nakaya N., Homa Y., Goto Y (1988). Cholesterol lowering effect of Spirulina. Nutr Rep Int. 37: 1329-1337.

Nascimento L.F.C., Botura Jr., G. & Mota R.P. (1996). Crescimento de E. coli submetida a campos magnéticos. In: Encontro Nacional de Física da Matéria Condensada, 19., Águas de Lindóia. Resumos. pp. 281.

O. Pulz., (2001). Photobioreactors: production systems for phototrophic microorganism. Appl. Microbiol Biotechnol. 57: 287-293.

Ogava T., and Terui G., (1970). Studies on the growth of Spirulina platensis on the pure culture of Spirulina platensis. J. Ferment. Technol. 48: 361-367.

Ogawa T., Aiba S., (1977). Assessment of growth yield of a blue-green alga Spirulina platensis in axenic and continuous culture. J Gen Microbiol. 102:179-182.

Ogbonna JC., Tanaka H., (1997). Industrial-size photobioreactors. Chemtech. 27: 43-49.

Others S., Pire R. (2001). Fatty acid composition of Chlorella and Spirulina microalgae species. J. AOAC Int. 84: 1708-1714.

Paredes-Carbajal MC, Torres-Duran PV, Diaz-Zagoya JC, Juarez-Oropeza MA (1997). Effects of dietary Spirulina maxima on endothelium dependent vasomotor responses of rat aortic rings. Life Sc. 1997;(61): 211-19.

Pradeepa R, Deepa R, Mohan VJ (2002). Epidemiology of diabetes in India-current perspective and future projections. Indian Med Assoc. 100(3): 144-8.

Rahimi R., Nikfar S., Larijani B., Abdollahi M. (2005). A Review on the antioxidants in the management of diabetes and its complications. Biomedicine & Pharmacotherapy 59:365-373.

Rao DLN (1979). The studies of mineral nutrition , mass cultivation and nutritional value of blue green algae S.platensis , Geitler , Ph.D . Thesies , IARI , New Delhi -47.

Raoof B, Kaushik BD, Prasanna R (2006). Formulation of low cost medium for mass production of Spirulina. Biomass and bioenergy 30 , 537- 542.

Richmond A (1992) Open system for mass production of photoautotrophic microalgae outdoors: Physical principles. J. appl. Phycol. 4: 281–286.

Richmond A., Boussiba S., Vonshak V., Kopel R. (1993). A new tubular reactor for mass production of microalgae outdoors,J. Appl. Phycol. 5: 327-332.

Richmond A., (1992). Mass culture of cyanobacteria. In: Mann, N., Carr, N., Eds. Photosynthetic prokaryotes. 2nd ed. Plenum Press, New York and London. pp. 181-210.

Sassano CEN, Gioielli LA, Almeida KA et al (2007) Cultivation of Spirulina platensis by continuous process using ammonium chloride as nitrogen source. Biomass Bioenerg 31: 593-598

Seber CAF and Wild CJ (2003). Nonlinear Regression. Wiley Interscience, 30-32.

Shekharam K., Ventakaraman L., and Salimath P., (1987). Carbohydrate composition and characterization of two unusual sugars from the blue-green algae Spirulina platensis. Phytochem. 26:2267-2269.

Singh, S.S., Tiwari, S.P., Abraham J., Rai, S., and Rai, A. K., (1994). Magnetobiological effects on a cyanobacterium, Anabena doliolum. Electro-and Magnetobiology., 13, 227-235.

Slinkard K, Singleton VL, (1977). Total phenol analysis automation and comparison with manual methods American journal of Enology and viticulture 28, 49- 55.

Soletto D, Binaghi L, Lodi A et al (2005). Batch and Fed Batch cultivations of Spirulina platensis using ammonium sulphate and urea as nitrogen sources. Aquacult 243: 217 – 224

Takahashi F., Kamezaki T (1985). Effect of magnetism of growth of Chlorell. Hakkokagaku. 63: 71-74.

Takai Y., Hossayamada Y., Kato T (1991). Effect of water-soluble and water-insoluble fractions of Spirulina over serum lipids and glucose resistance of rats. Journal of Japan Society Nutrition Food Science. 44: 273-277.

Tang L ,Wei ,Chen L,Liu S., (2006). Effects of Berberine on diabetes induced by alloxan and a high-fat\high cholerstrol diet in rats, Journal of Ethnopharmacology 108,109-115.

Tanticharoen M, Reungjitchachawali M, Boonag BP et al (1994). Optimization of γ-linolenic acid (GLA) production in Spirulina platensis. J Appl Phycol. 6: 295–300.

Tomaselli, L (1997). Morphology, ultrastructure and taxonomy of Arthrospira (Spirulina) maxima and Arthospira (Spirulina) platensis. In: Vonshak, A., Ed. Spirulina platensis (Arthrospira): Physiology, cell-biology and biotechnology. Taylor and Francis. London. pp. 1 – 16.

Torre P, Sassano CEN, Sato S, Converti AL et al (2003). Fed-batch addition of urea for Spirulina platensis cultivation-Thermodynamics and material and energy balances. Enzyme microbial Technol 33: 698 –707.

Torres-Duram P.V., Miranda-Zamora R., Paredes-Carbajal M. C., Mascher D., Diaz-Zagoya J.C., Juarez-Oropeza M.(1998). Spirulina maxima prevent induction of fatty liver by tetrachloride in the rat. Biochemistry Molecular Biology International. 44: 787-793.

Torzillo G., Carlozzi P (1996). Productivity of Spirulina in a strongly curved outdoors tubular photo bioreactor. Appl Microbiol. Biotechnol. 45:18-23.

Tredici MR., Carlozzi P., Chini Zitteli G., Materassi R. (1991). A vertical alveolar panel (VAP) for outdoor mass cultivation of microalgae and cyanobacteria. Biores. Technol. 38: 153-159.

Tripanji , Suharyanto (2001) Optimization medium from low-cost nutrient sources for growing Spirulina platensis and carotenoids production. Menara Perkebunan 69 (1), 18-28.

Tuschil H., Neubauer G., Schmid G., Weber E., & Winker N (2000). Occupational exposure to static, ELF, VF and VLF magnetic fields and immune parameters. Int. J. occup. Med. environ. Hlth. 13: 39-50.

Van Eykelenburg C., (1977). On the morphology and ultra structure of cell wall of Spirulina plantensis Antonie Van Leewenhoek. 43: 89-99.

Venkatraman L. V., Bhagyalakshmi N., Ravishankar G. A.(1995). Commercial production of micro and macro algae problems and potential. Indian Journal of Microbiology; 35:1-19

Vonshak A., and Tomaselli L (2000). Arthospira (Spirulina): Systematics and ecophysiology. In: Whitton A., Potts M., Eds. The Ecology of Cyanobacteria. Kluwer Academic Publishers. The Netherlands: pp. 505-522.

Vonshak A., (1997). Appendices. In: Vonshak, A., Ed. Spirulina platensis (Arthrospira): Physiology, Cell biology and Biotechnology. Taylor and Francis, London, Great Britain; pp. 213- 226.

Vonshak A., Chanawongse L., Bunnag B., Tanticharoen., (1988). Light acclimation and photoinhibition in three Spirulina platensis (cyanobacteria) isolates. J. Appl, Phycol. 8: 35-40.

Vonshak A., Richmond A (1988). Mass production of blue-green algae Spirulina- an overview. Biomass. 15: 233-47.

Yamoakas Y., Takimura O., Fuse H., and Kamimura K (1992). Effect of magnetism on growth of D. naliella salina. Research in Photosynthesis, 3: 87-90.

Zarrouks C (1966) Contribution a' l'etude d'une cyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de Spirulina maxima. Ph.D Thesies, Universite' de Paris , Paris.

Zeng AP and Deckwer WD (1995) A kinetic model for substrate and energy consumption of microbial growth under substrate-sufficient conditions. Biotechnol Prog 11: 71–79.

UTILITY OF EQUIPMENT

Besides research use, the Air-lift photo bioreactor and HPLC are open to M.Sc Biotechnology & Biochemistry streams of student for laboratory exercises.

ACKNOWLEDGEMENTS

1 members of expert committee of MP Biotech Council

Chief Executive Officers

2. Shri R.B.Sinha

3. Shri.S.K.Panwar

4. Shri.Saibal Das Gupta

5. Adviser: Dr.Moni Mathur