

Isolation and identification of UV absorbing compounds from cyanobacteria (*Synechocystis*) of District Mardan

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Abstract- Blue green algae (also known as cyanobacteria) are photosynthetic prokaryotes found in a variety of environments. They may be aquatic, epiphytic, rhizospheric, endophytic, found in fresh water or salty water or on damp soil. Biotechnological applications of cyanobacteria are many including their use as a source of UV absorbing pigments. During current study, *Oscillatoria*, *Synechocystis*, *Gloeocapsa*, *Nostoc*, *Cyanothece* and *Microcystis* were isolated from different locations of Gujjar Garhi using a modified version of BG11. The strains were identified by colony morphology and light microscopy. The isolates were grown in the presence of tube light and sunlight and screened for UV absorbing compounds. Though all the strains produced UV absorbing compounds but only negligible or no absorbance in the UV-A and UV-B regions was recorded. Being the most harmful component of UV light due to their ability to reach the earth atmosphere, our aim was to induce production of compounds in the isolated cyanobacteria which can screen these components of UV radiation. Exposure to UV light was an excellent force inducing the isolates to produce UV-A and UV-B absorbing compounds. All the isolates produced significant quantities of phenols and flavonoids in both sunlight

and tube light. However, *Synechocystis*, *Gloeocapsa* and *Oscillatoria* produces higher quantities of flavonoids than *Cyanothece*, *Nostoc*, and *Microcystis*. Production of phenols and flavonoids is greatly induced upon exposure of the isolated cyanobacteria to UV. Phenols and flavonoids are important for their role in the antioxidant system of cyanobacteria. Additionally, they also absorb significantly in the UV-A and UV-B regions as HPLC fractions containing phenols or flavonoids were efficient in absorbing in these regions. It may be concluded that cyanobacterial isolates naturally produce UV-C absorbing compounds, however UV-B and UV-A absorbing compounds induced by UV light exposure. Absorbance in these regions is positively correlated with the production of phenols and flavonoids which are induced by exposure to UV light.

Index Terms- (Cyanobacteria, *Synechocystis*, UV absorbing compounds, UV Radiations, HPLC)

I. INTRODUCTION

Blue green algae are photosynthetic prokaryotes found in a variety of environments. They may be aquatic, epiphytic, rhizospheric and endophytic. These cyanobacteria can be found in fresh water, marine water and in moist places. They are small

creature include unicellular, colonial or filamentous organisms. They are important companion of higher plants undergoing symbiotic association with a number of higher plants, providing their hosts with nitrogen and other nutrients or phytohormones, as we know that cyanobacteria are atmospheric nitrogen fixers thus they transfer it into their partner where both partners are benefited (Whitton et al., 2012). These blue green algae are important to aquatic biology of both fresh and marine animals. Cyanobacteria convert sunlight by photosynthesis to release oxygen into water. They are also important in health and food industry. Algae are the most useful organism in the world as they consume most of CO₂ from the air and release most amount of oxygen which keep us alive and are being used in many industries and applications. These tiny algae are important source of algal biofuels. Besides, other important products of cyanobacteria include carotenoids, phycobilins, fatty acids, vitamins, sterols and many bioactive compounds, which have been used for human health and in pharmaceuticals. Anti-cellulite and Alguronic acid are also extracted from algae which are used in cosmetics. Antivirals and antifungals are also present in most of cyanobacterial species which are being currently commercialized and used in pharmaceutical industries.

UV radiations has both the beneficial and harmful effects to human health. UV radiation is responsible for bone strengthening because they produce the bone strengthening vitamin D. It seems that those

who get higher sun there are more vitamin D in their body and those who get lower sun there are less vitamin D. Vitamin D is known to control calcium metabolism in human and hence play important role in bone strengthening and growth (Backer et al., 2013). On the other hand, over-exposure to ultra violet radiation is associated with sun burn, freckling and cancer etc. Also, ultra violet photons harm the DNA molecules of living organism which results in harmful modifications in genes. Peoples with missing lens or replacement lens can see some ultra violet wavelengths (Hambling et al., 2002). It has been recognized many years ago that UV-B radiation cause skin crythema (Skin burn) and prolonged exposure to UV-B radiation can lead to DNA damage, immunosuppression and sometimes may also lead to cancer (Boelen et al., 2002).

UV radiations can also effect algae and cyanobacteria (blue green algae). Many biochemical and biological process which has been reported to be effected by UV radiation include nitrogen metabolism, carbon dioxide uptake, photosynthetic O₂ production and growth (Sinha et al., 2007). Moderate ultraviolet radiation effect photosynthesis and N₂ fixation in *Anabaena* species (Tedett et al., 2006). Ultraviolet radiations mainly divided into three regions including UV-A, UV-B and UV-C. UV-B radiation generally decrease chlorophyll content and photosynthesis in cyanobacteria which results in lower biomass. UV-B radiation can damage several biological and biochemical processes in cyanobacterial cells.

Different organisms produce different types of compounds which protect them against the harmful UV radiation. Cyanobacterial (Blue green algae) are known to produce a number of secondary metabolites and pigments which protect their cells against the harmful influence of UV radiation.

In cyanobacteria, a number of ultraviolet (UV) absorbing compounds including mycosporine amino acid and Scytonemin are found which help them grow in the presence of UV radiation. Mycosporine like amino acids are ultra-violet absorbing compounds which are photo protective agent against-UV radiation in aquatic organisms (Rastogi et al., 2010). MMA (Mycosporine- amino acids) absorb ultraviolet radiation in the range of 310-360 and believed to acting as sunscreens. Cysteine type of MMA is produced in most of marine organisms (Sinha et al., 1998). MMA are small (≤ 400 Da). They have no color and are water soluble and are characterized by cyclohexanone. They have very strong ultra-violet absorption in the range of 310 and 362 nm. In water soluble components the cyclohexanone and cyclohexeniminechromophor is UV captivates. Different amino acids incorporates results in a variety of twenty micosporine amino acids (Cockell et al., 2000). Cyanobacterial carotenoids such as lutein, caloxanthin, oscillaxanthin, canthexanthin and polyphenolic compounds such as Scytonemin and mycosporine amino acid are used in sun blocking cream. Several natural and synthetic products are used to make sunscreens which are

applied to human skin in order to avoid UV damage. Sun screens is a type of gel, lotion, spray that absorbs sun harmful radiations or reflect these UV rays from the skin of humans exposed to sunlight. These sunscreens contain UV absorbing substances used for the human's skin to protect them from harmful UV radiations (Lowe et al., 1997). Substances used in these sunscreens may be organic or inorganic. Organic substances include benzophenones, triazines, benzotriazoles and methoxydibenzoylmethane, while inorganic substances include titanium dioxide and zinc oxide (Zhang et al., 1998).

Current project was designed to screen local cyanobacteria for UV absorbing compounds and identify such compounds. For this purpose local cyanobacteria were isolated, cultured and screened for absorbance in UV-A and UV-B regions. HPLC fractionation and LC-MS/MS analysis was then performed to identify the compounds involved in absorbing the harmful UV radiation.

II. MATERIAL AND METHODS

A. *Sampling Cyanobacteria*

Different samples of cyanobacteria were collected randomly from moist places and ponds present in district Mardan. Forceps were used to collect algal specimens and stored at 4 °C for further experiments.

B. *Light Microscopy*

Microscopy of samples were done with the help of a light microscope. The slides were then observed under 10x, 40x and 100x objective lenses to identify the specimens.

C. Ultra violet scanning

Supernatant of both BG11 and biomass extract in acetone were scanned for absorbing radiation in the range of 200 nm to 800 nm using a UV/vis spectrophotometer (PerkinElmer lambda 25 spectrophotometer).

D. Samples incubation in sun and tube lights

Samples were grown in the presence of sun light and tube light to evaluate the effect of different light sources on the growth and production of UV absorbing compounds by the isolates. The supernatant and biomass crushed acetone were scanned for absorbing in the UV range of the spectrum at intervals of 1 week, two weeks and three weeks of incubation.

E. Cyanobacterial growth under UV light

Samples were grown in broth for two weeks and then exposed to strong ultra violet radiation for different duration of time i.e. for one hour, three hours, six hours and twenty four hours.

F. Growth measurement

Growth measurement was done after incubating the samples in sun light and tube light for three weeks. The cultures were centrifuged at 14500 RPM for ten minutes, the supernatant were discarded and the pellets were weighed.

G. Flavonoids determination

Flavonoids were determined in the cultures of isolated cyanobacteria by using colorimetric assay as described by Lillian et al, (2007). The quantity of flavonoids in the samples were calculated from standard curves and were expressed in mg of equivalent catechol/g.

H. Phenol Determination

Phenols were determined in the cultures of isolated cyanobacteria. Folin reagent were used as a blank. Their OD were recorded at 650 nm.

A. Isolation and characterization of algae

Synechocystis was found attached to a substratum in fresh water were isolated. Isolation and purification were carried out

I. Statistical Analysis

Data was analyzed statistically for significance using ANOVA and Duncan multiple range test ($p = 0.05$) through SPSS for windows ver 16.0.

J. HPLC fractionation

The samples showing high absorbance in the UV-A and UV-B regions were subjected to fractionation by HPLC equipped with a reverse phase column, isocratic pump and fraction collector. All the peaks were collected and stored for further processing at 4 C.

K. LC-MS

For the identification of UV absorbing compounds, fractions of HPLC were subjected to ESI-MS/MS (LTQ XL, Thermo Electron Corporation, USA), for this purpose direct injection method were used (Steinmann et al., 2011). Methanol and acetonitrile at the ratio of [80:20 (v/v)] were used as a solvent. Mass range in ionization mode were selected from 50 to 1000 m/z. on the basis of parent molecular ion nature collision dissociation energy were kept in range of 10-45 during MS/MS. Temperature of capillary were kept at 280 °C whereas sample flow rate were regulated to 8 μ L/min. to study ionization, ion transfer and optimum signal, MS parameter for each compound were adjusted. This process were done through filling analytes and rotating parameters. For all the analytes source parameters were same. The acquired ESI-MS/MS data was analyzed using manual, Xcalibur (Xcalibur 3.0). ChemDraw (ChemDraw Ultra 8.0) software was used for structural elucidation and then compared with online published data.

III. RESULTS

through a two steps procedure. Firstly, samples of algae were spreaded on plates and then streaked for purification. Algal growth appeared on plates after incubation of 10-15 days at

room temperature. Cyanobacterial growth were appeared after 20-25 days. Tentative analysis of their morphology was done. Purified strain were grown in BG11 broth for 25 days. After sufficient biomass in broth strain were then grown in sunlight and tube light for different i.e 1 week, 2 weeks and three to check effect of incubation time on isolated strain. After every week their supernatant were scanned to check UV absorbing compounds, to measure growth and secondary metabolites. Similarly isolated strain were also grown under UV light for different duration of time i.e for 1, 3, 6 and 24 hours to check effect of UV light on growth, UV absorbing compounds and secondary metabolites.

B. Light microscopy

Unicellular *Synechocystis* sp SC1 were observed under light microscope (Figure 4.1). It was observed that *Synechocystis* sp SC1 have three dimensional structure and spherical unicells without mucilaginous sheath. Cells of *Synechocystis* sp SC1 form colonies.



Fig 4.1 light microscopy of *Synechocystis*

Now it is the time to articulate the research work with ideas gathered in above steps by adopting any of below suitable approaches:

C. UV Absorbance of *Synechocystis*

Synechocystis sp SC1 was grown in tube light and sunlight for one week and the culture supernatant and acetone extract of biomass were scanned for absorbance in different regions of UV including UV-A, UV-B and UV-C using a spectrophotometer. Culture supernatant obtained from culture

grown in tube light for a week showed absorbance in the UV-C region. Absorbance in the UV-A and UV-B regions was only negligible (Figure 4.3A). In UV-B region absorbance peaked (0.49) at 286 nm, while in UV-A region greatest absorbance recorded was (0.46) at 334 nm (Figure 4.3A). Acetone extract of the biomass had absorbed radiation in UV-B and UV-A regions. In UV-B region greatest absorbance was (2.05) at 299 nm. There was no absorbance in the UV-A region (Figure 4.3B). Culture supernatant of *Synechocystis* sp SC1 grown in the presence of sun light showed similar absorbance pattern as was noted in case of sunlight (Figure 4.3A). However, the acetone extract of biomass showed less absorbing pattern as compared to the tube light grown cultures. Less absorbance in the UV-C region was recorded. In UV-A region the absorbance peaked (0.936) at 320 nm (Figure 4.3B). In UV-B region, the greatest absorbance recorded was (0.36) at 388 nm.

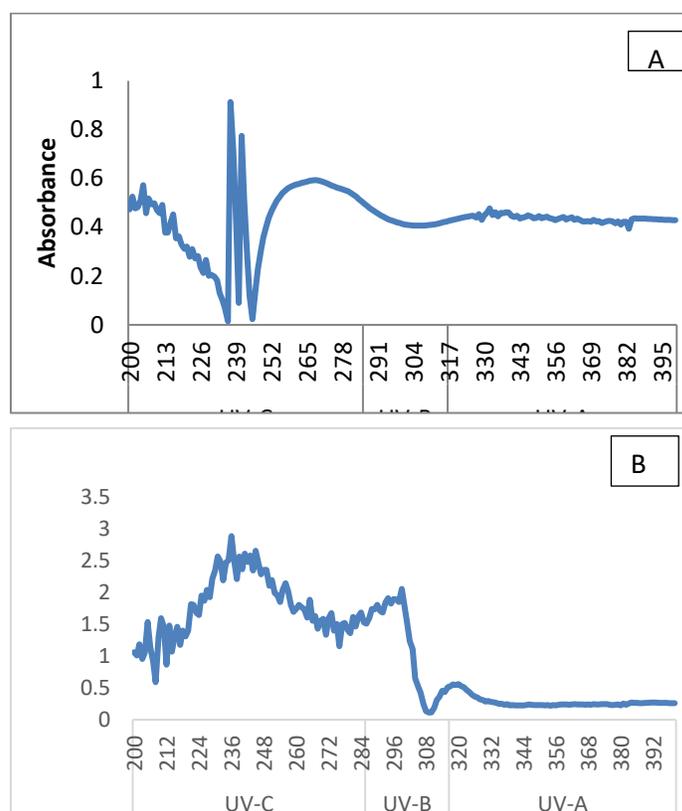


Figure 4.2. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 1 week exposure to tube light.

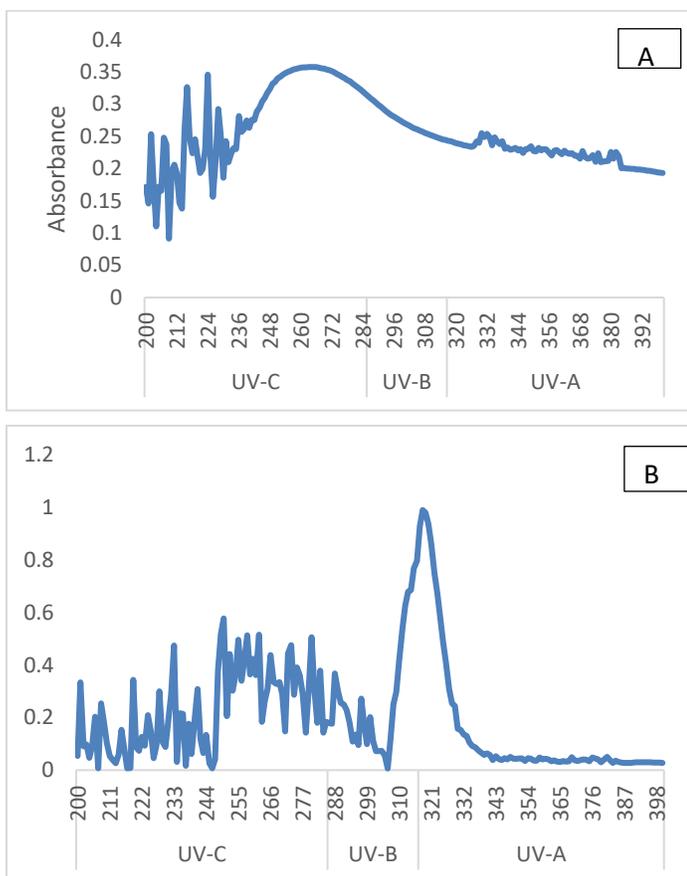


Figure 4.3. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 1 week exposure to sun light

To check the effect of incubation time on UV absorbance, culture of *Synechocystis* sp SC1 grown for two weeks was subjected to UV absorbance scan. It can be seen that culture supernatant of tube light exposed cultures of *Synechocystis* sp SC1 produced an absorbance scan quite different from the one week old cultures. Less absorbance were noted in UV-C region. There were no absorbance in UV-B and UV-A regions (Figure 4.4A). Similarly, acetone extract of the cyanobacterial biomass also showed different pattern of absorbance. The greatest absorbance was noted in UV-C region. In UV-B region, absorbance peaked (0.55) at 281 nm (Figure 4.4B). This absorbance was clearly different from the absorbance pattern noted for culture allowed to grow for one week only. Culture supernatant of sun light exposed cultures of *Synechocystis* sp SC1 produced an absorbance scan similar to tube light exposed culture (Figure 4.5A). Acetone extract of the cyanobacterial biomass showed greatest absorbance in UV-C and UV-B regions. In UV-B regions the greatest absorbance recorded was

(6.3) at 289 nm as compared to *Synechocystis* sp SC1 culture allowed to grow for one week only.

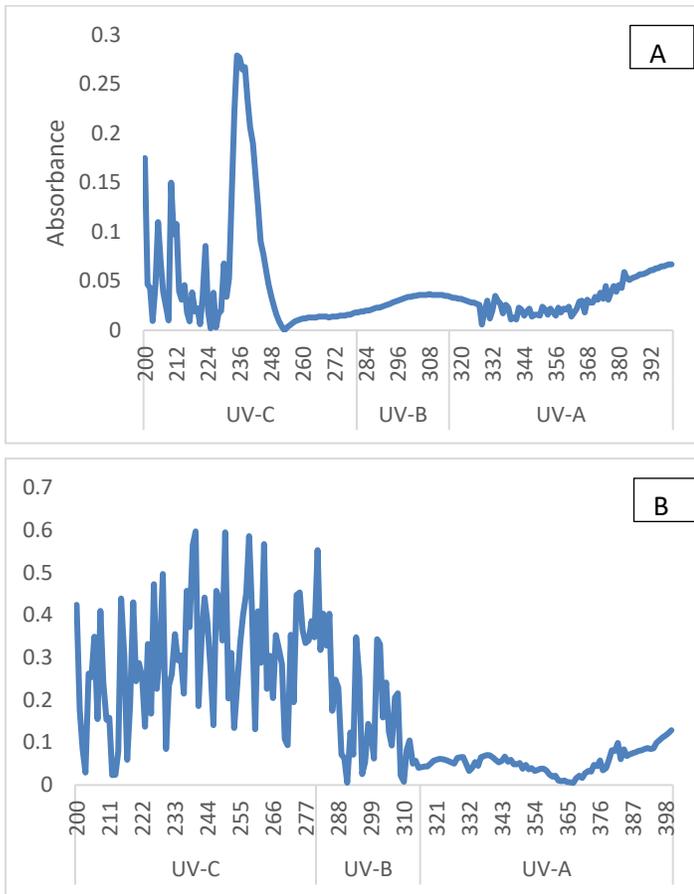


Figure 4.4. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 2 weeks exposure to tube light.

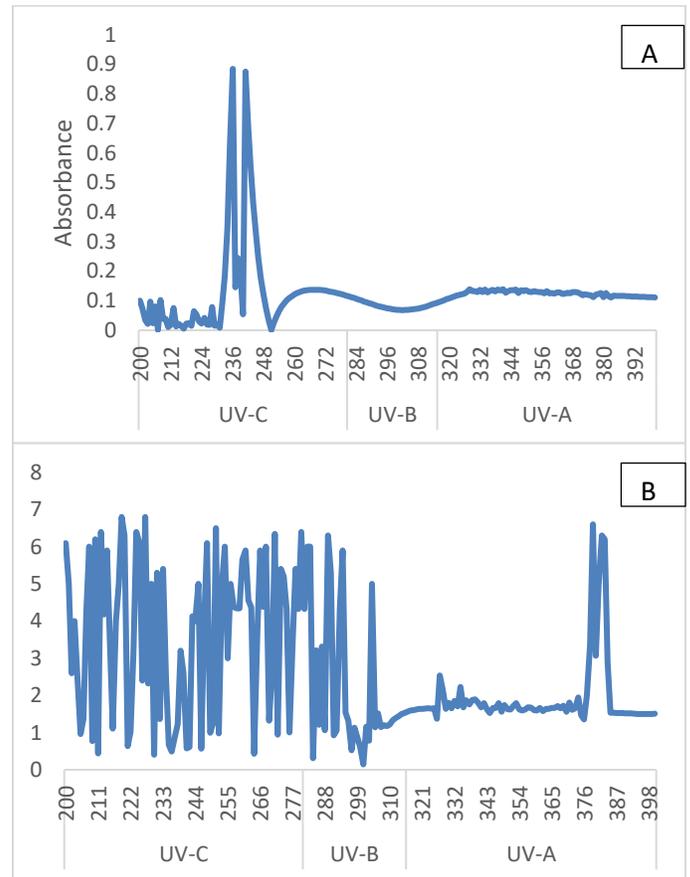


Figure 4.5. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 2 weeks exposure to sun light.

Synechocystis sp SC1 Culture grown for three weeks subjected to UV absorbance to check the effect of incubation time. It can be seen that culture supernatant of tube light exposed cultures of *Synechocystis* sp SC1 produced an absorbance scan different from one week and two weeks old cultures (Figure 4.6A). Interestingly, acetone extract of the cyanobacterial biomass showed less absorbance in UV-C region only and no absorbance were recorded in UV-A and UV-B regions of spectrum. Culture supernatant of *Synechocystis* sp SC1 grown in the presence of sun light showed similar absorbance pattern as compared to one week old cultures (Figure 4.7A). However, the acetone extract of biomass showed less absorbing pattern as compared to the one and two week's old cultures (4.7B). In sun light grown culture, most of the absorbance was in the UV-C region of UV (Figure 4.7B). In UV-B region, the greatest absorbance recorded was (0.41) at 314 nm. In the UV-A region the absorbance peaked (0.79) at 316.

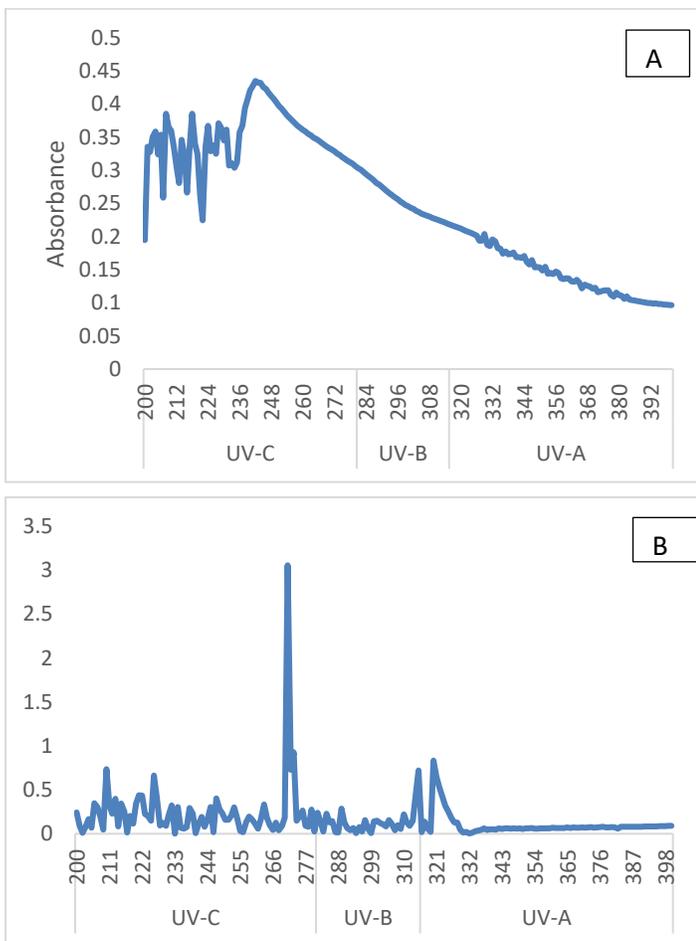


Figure 4.6. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 3 weeks exposure to tube light.

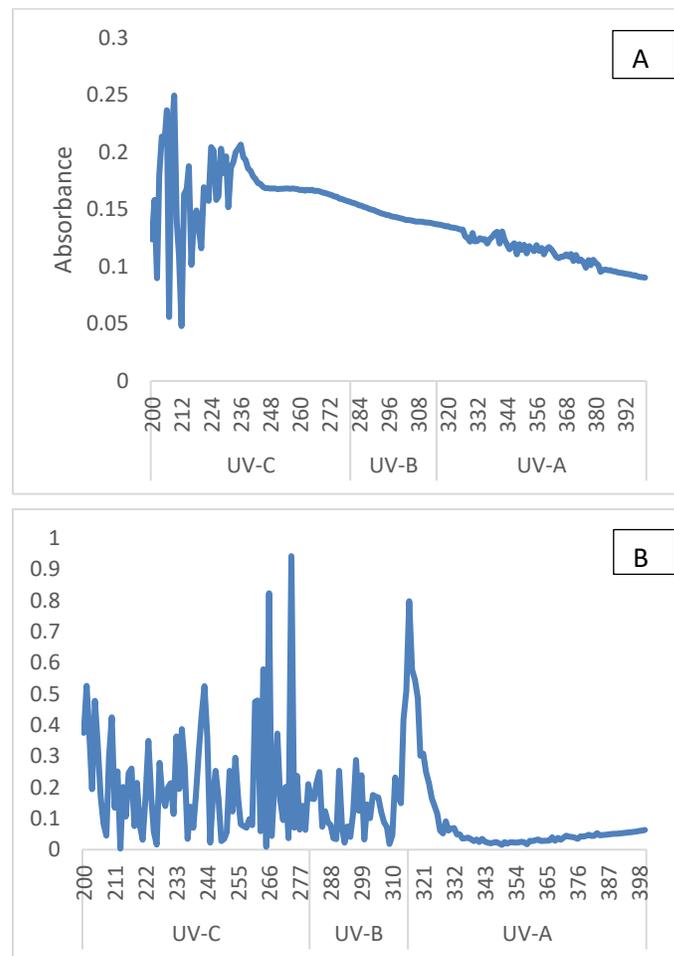


Figure 4.7. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 2 weeks exposure to sun light.

Effect of UV light on *Synechocystis*'s ability to absorb UV light

Synechocystis sp SC1 was exposed to UV light for different duration of time i.e for 1 hour, three hours, six hours and twenty four hours, culture supernatant and acetone extract of biomass were scanned for absorbance in different regions of UV including UV-A, UV-B and UV-C using a spectrophotometer. Culture supernatant obtained from culture grown in UV light for one hour showed absorbance in the UV-C region. In UV-B and UV-A regions no absorbance were recorded (Figure 4.8A). Acetone extract of the biomass had absorbed radiation in all the three regions of UV (Figure 4.8B). In UV-B region, the greatest absorbance recorded was (6.57) at 290 nm and in UV-A region absorbance peaked (7.01) at 328 nm. Culture supernatant of UV light exposed cultures for three hours produced an absorbance

scan were similar pattern to the one hour old cultures (Figure 4.9A). Acetone extract of the biomass had absorbed radiation in all the three regions of UV (Figure 4.9B). In UV-B region, the absorbance peaked 6.9 at 308 nm and in UV-A region greatest absorbance recorded was 7.40 at 350 nm (Figure 4.9B). Culture supernatant obtained from culture grown in UV light for six hour produced an absorbance scan different from the one hour and three old culture (Figure 4.10A). Absorbance in UV-C region were recorded maximum, while in UV-B and UV-A no absorbance were recorded (Figure 4.10A). Acetone extract of the biomass had absorbed radiation in all the three regions of UV (Figure 4.10B). In UV-B region absorbance peaked (7) at 310 nm, while in UV-A region greatest absorbance was (7) at 330 nm. To check the effect of incubation time on UV absorbance, culture of *Synechocystis* sp SC1 grown for 24 hours under UV light was subjected to UV absorbance scan. Culture supernatant produced an absorbance scan similar to 3 hours old cultures (Figure 4.11A). Acetone extract of the biomass had absorbed radiation. In UV-B region greatest absorption recorded was (6.9) at 308 nm, while in UV-A region the absorbance peaked (7.40) at 350 nm.

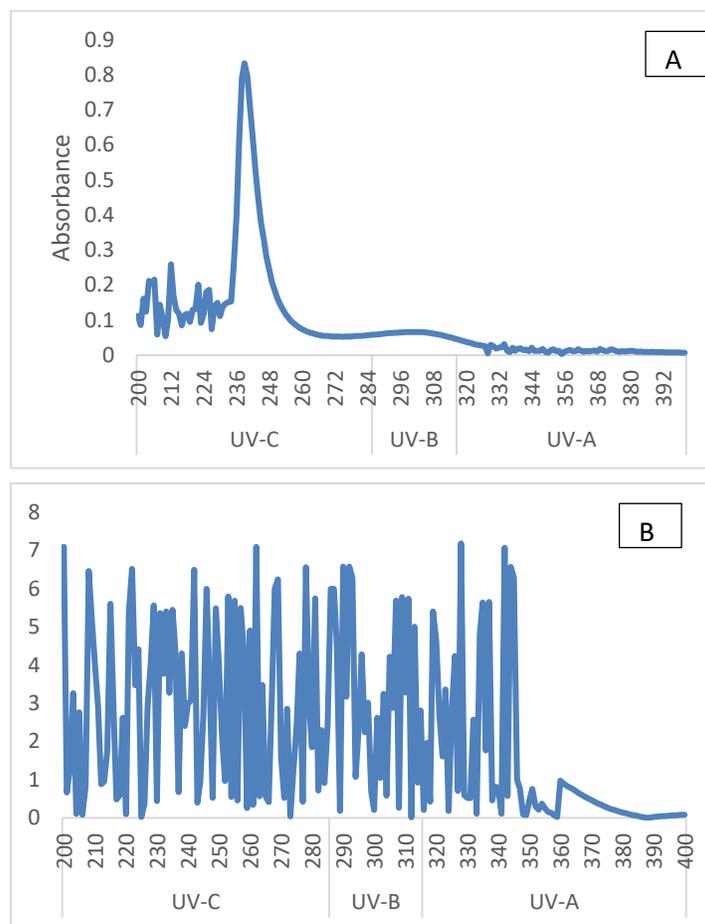


Figure 4.8. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 1 hour exposure to UV light.

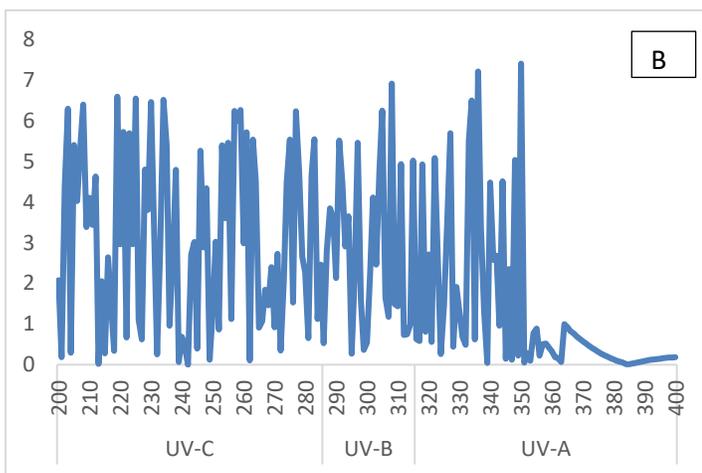
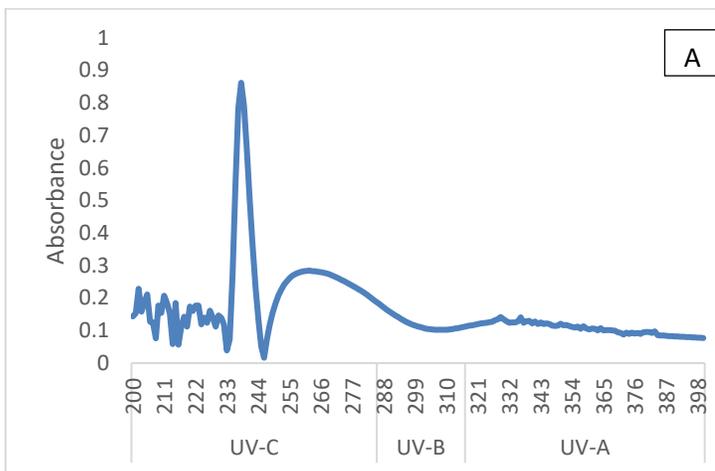


Figure 4.9. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 3 hours exposure to UV light.

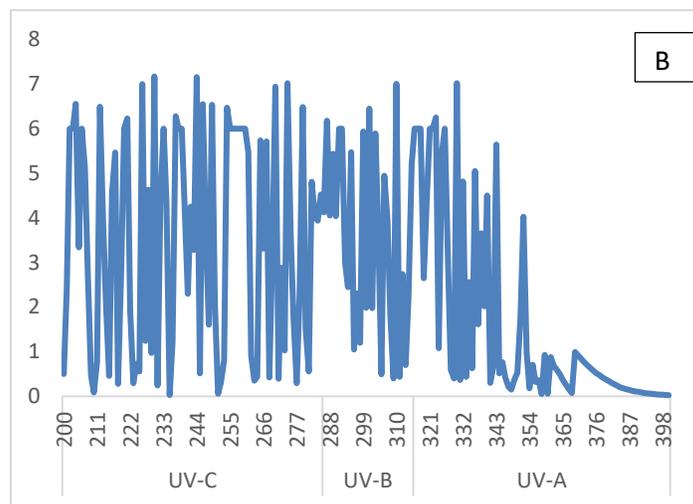
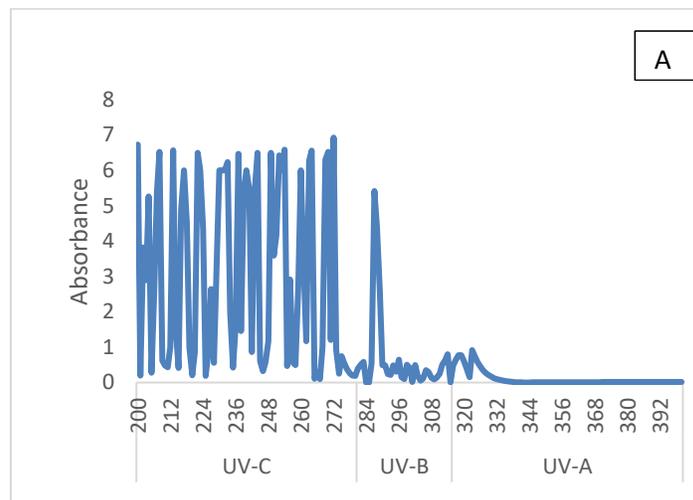


Figure 4.10. Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 6 hours exposure to UV light.

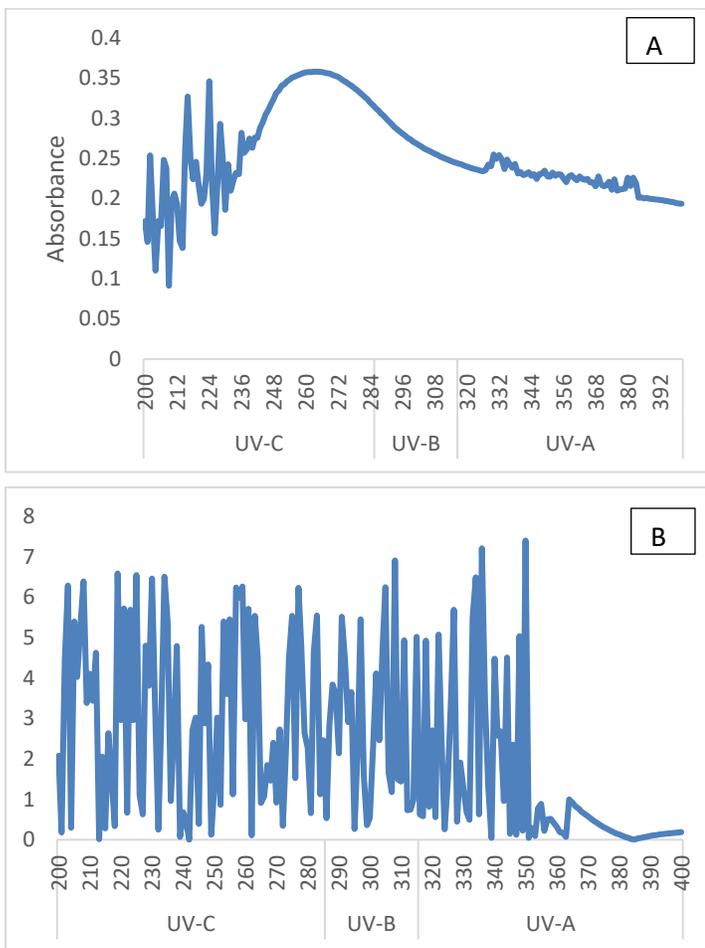
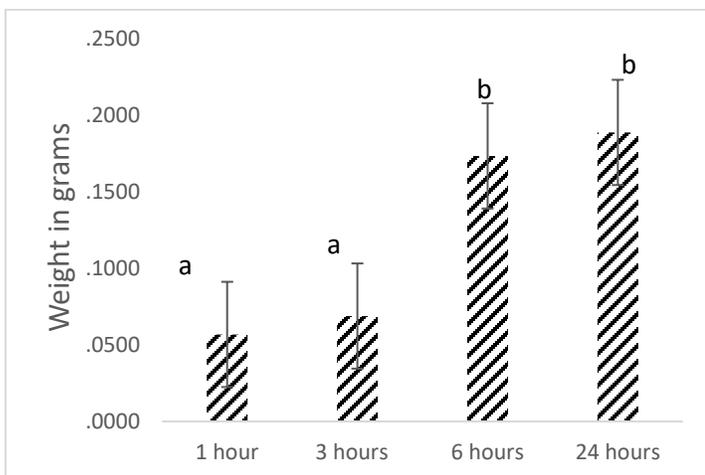


Figure 4.11 Absorbance in the UV-C, UV-B and UV-A region by *Synechocystis* sp SC1 culture supernatant (A) and acetone extract of its biomass (B) after 24 hours exposure to UV light.

Effect of different sources of light on the growth of cyanobacteria



After one week exposure of sun light and tube light, growth of *Synechocystis* sp SC1, were measured by using balance. Weight of the strains was recorded as mg mL⁻¹. For *Synechocystis* sp SC1 sun light was good source of light and the strain produced up to 2-fold more biomass than one week (Figure 4.12). Maximum growth were recorded in sun light grown *Synechocystis* sp SC1. After three weeks the biomass were decreased in *Synechocystis* sp SC1.

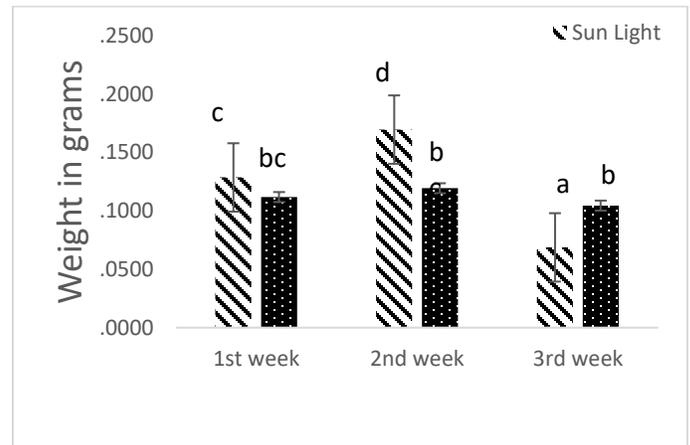


Fig 4.12. Measurement of growth in *Synechocystis* sp SC1 after three weeks. Different labels on the bars of same color indicate significant difference ($p < 0.05$; Duncan test).

Growth of *Synechocystis* sp SC1 was measured after exposure to UV light for different duration i.e, for one, three, six and twenty four hours. Weight of the strains was recorded as mg mL⁻¹. In case of *Synechocystis* sp SC1 UV light was good source of light and their biomass increased gradually when exposed to UV for different duration of time (4.13).

Fig 4.13. Effect of UV exposure on the growth of *Synechocystis* sp SC1. Different labels on the bars indicate

significant difference between means ($p < 0.05$; Duncan test).

Production of flavonoids by cyanobacteria

Synechocystis sp SC1 was grown in the presence of sunlight and tube light for different duration of time i.e for one week, two weeks and three weeks and then amount of flavonoids released in the cultures was measured. Flavonoids released were sharp (Figure 4.14). To check the effect of incubation time on flavonoids released, cultures were monitored after two weeks. Maximum production of flavonoids were recorded in *Synechocystis* sp SC1 (Figure 4.14). Gradual increase occurred in the production of flavonoids for two weeks. After three weeks determination of flavonoids were done *Synechocystis* sp SC1 produced higher quantities of total flavonoids when grown in tube light than the sun light culture (Figure 4.14).

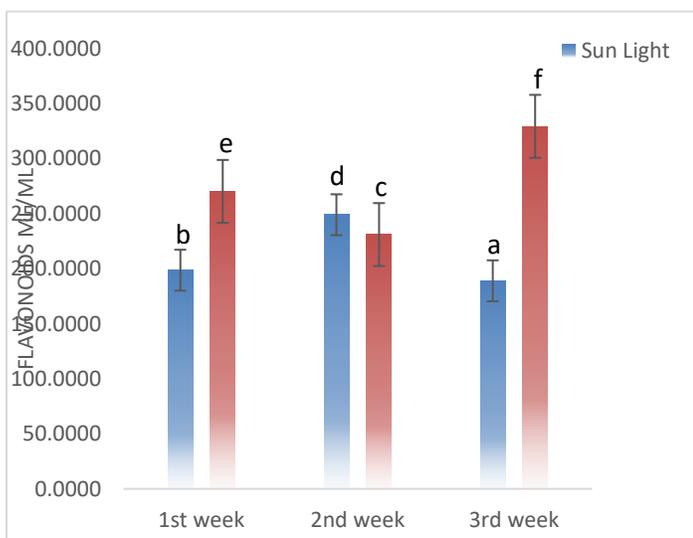


Fig 4.14. Determination of flavonoids in *Synechocystis* sp SC1 culture exposed to tube light and sun light for one week. Flavonoids were determined by colorimetric method after one week. Different labels on the bars of same color indicate significant difference ($p < 0.05$; Duncan test).

Synechocystis sp SC were exposed to UV light for different duration of time i.e, for 1 hour, 3 hours, six hours and twenty four hours and then flavonoids released in the culture were determined by colorimetric method. There were gradual time dependent increase in the amount of flavonoids released into the *Synechocystis* sp SC1 for six hours and after six hours their

flavonoids concentration were same when exposed to UV light for twenty four hours (Fig 4.15).

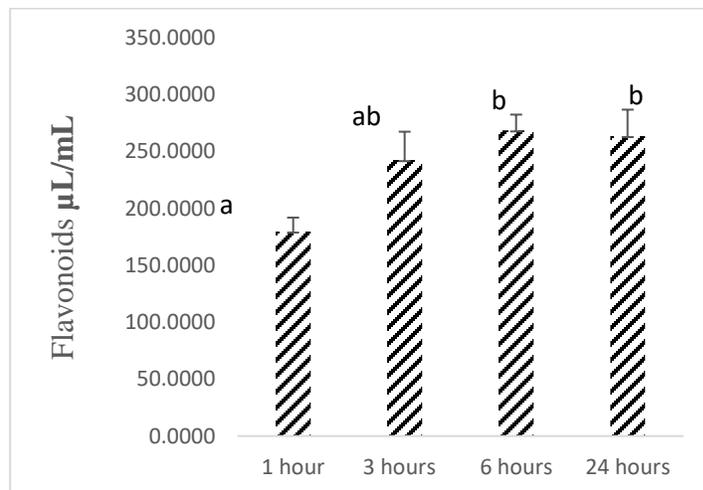


Fig 4.15. Effect of UV exposure on flavonoids of *Synechocystis* sp SC1. Different labels on the bars indicate significant difference between means ($p < 0.05$; Duncan test).

Production of Phenol by cyanobacteria

Synechocystis sp SC1 were grown in the presence of sunlight and tube light for different duration of time i.e, for one week, two weeks and three weeks and then production of phenols in the cultures was measured. Sunlight was the most suitable source of light for greater production of phenols by *Synechocystis* sp SC1. After two weeks quantity of phenol in the cultures were measured. *Synechocystis* sp SC1 produced higher quantities of total phenols when grown in sun light than the tube light cultures (Figure 4.16). To check the effect of incubation time on the production of phenols strains were grown in sunlight and tube light for three weeks (Fig 4.16). Decrease in the production of phenols in all cultures were monitored (4.16).

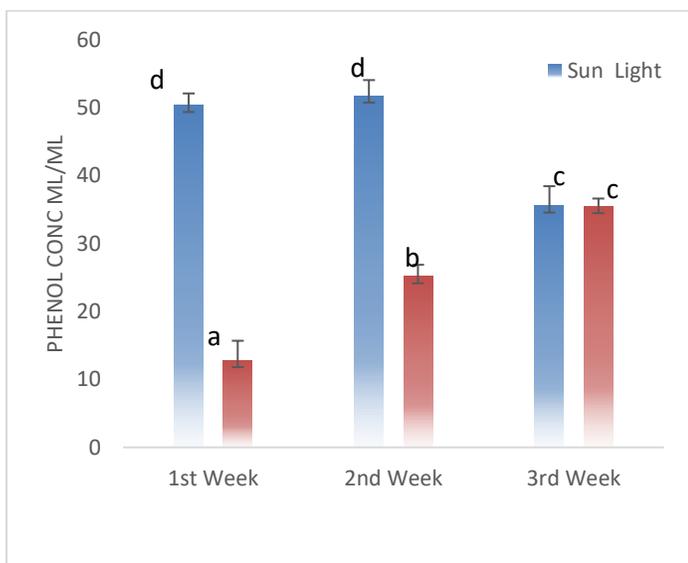


Fig 4.16. Determination of Phenol in *Oscillatoriasp* F1, *Synechocystis sp* SC1, *Gloeocapsa sp* S, *Nostoc sp* F2, *Cyanothece sp* SC2 and *Microcystis sp* SC3 cultures exposed to tube light and sun light after one week. Different labels on the bars of same color indicate significant difference ($p < 0.05$; Duncan test).

Synechocystis sp SC were exposed to UV light for different duration of time i.e, for 1 hour, 3 hours, six hours and twenty four hours and then phenol production in the culture were determined by colorimetric method. There were gradual time dependent increase in the amount of phenol released into the culture *Synechocystis sp* SC1 when exposed to UV light for 1 hour, 3 hours, six hours and twenty four hours (Fig 4.17).

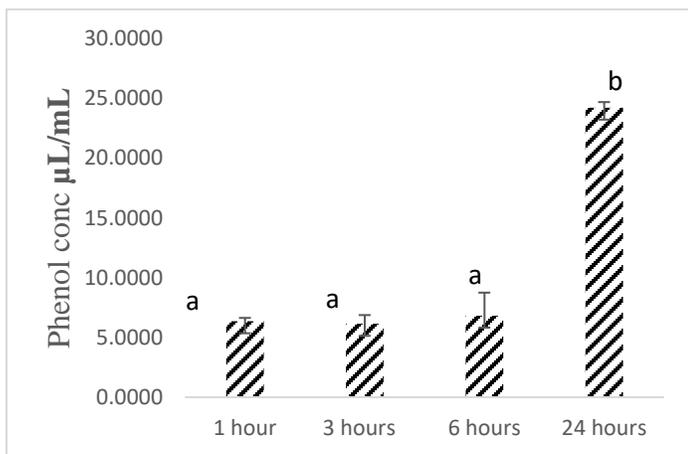


Fig 4.17. Effect of UV exposure on phenols of *Synechocystis sp* SC1. Different labels on the bars indicate significant difference between means ($p < 0.05$; Duncan test)

IV. DISCUSSION AND CONCLUSION

Synechocystis sp SC1 were isolated from gujar garhi Mardan. The isolated strain was cultured on modified version of BG11 medium. Strain were cultured for three weeks exposed to sun light and tube light and UV light. Growth of cyanobacterial strain were measured after 1, 2 and 3 weeks. Sunlight was the most suitable source of light for *Synechocystis sp* SC1 to grow optimally. In previous study, light emitting diodes (LEDs) with peak emittance of 680 nm was found to be the excellent source of light for enhanced productivity of microalgae by up to 2-fold without changing the cell volume (Carvalho et al., 2011). Light is the most important factor which control growth of cyanobacteria. Light quality have a dramatic effect on biomass production of cyanobacteria. Previously two cyanobacterial strains were studied to determine the effects of various wavelength distributions and irradiance levels on growth kinetics Red light produced the highest growth rate as compared to white light (Barnett et al., 2015). Response of cyanobacteria to the UV light was mixed. *Synechocystis sp* SC1 UV light was beneficial during initial exposure for 1, 3, 6 and 24 hours. To increase cell volume exposure of culture to white light was found suitable. Studies showed that growth of cyanobacteria is significantly affected by UV radiation. High UV-B radiations tend to decrease the chlorophyll contents of most of cyanobacteria. Also, exposure to UV-B has been shown to induce the production of absorbing compounds. Higher UV-B can also decrease the rate of photosynthesis. Decrease in chlorophyll contents and photosynthesis is subsequently followed by biomass production. However, algae and cyanobacteria have evolved various avoidance and repair mechanisms to protect themselves against the damaging effects of UV radiation (Xue et al., 2005).

Main objective of the current work was to monitor the ability of our isolates to absorb UV light. For this purpose, cultures exposed to different light sources including sunlight, tube light and UV radiation were tested in two ways. The culture supernatant was scan for UV absorbance in order to find extracellular UV absorbing compounds. Also, acetone extract from cyanobacterial

biomass was assayed which contained intracellular UV absorbing compounds. It was noted that most of the extracellular compounds were efficient in absorbing UV-C part of the spectrum, irrespective of the light source used. Tube light was most suitable source for the production of UV absorbing compounds. UV irradiation induces increase in extracellular UV absorbing compounds. Previously, it has been shown that exposure of a cyanobacterium *Nostoc sp F2 communeto* UV-A and UV-B irradiation enhanced the release of carotenoids, UV-A/B absorbing MAA which was correlated with glycan synthesis and extracellular Scytonemin. However, upon prolong exposure to UV-B release of scytonemin stopped whereas amount MMA's content remained high (Ehling *et al.*, 1999).

Most of the UV-A and UV-B absorbing compounds were retained intracellularly by the isolated cyanobacteria. It was noted that acetone extract of the biomass showed absorbance in all the three regions of UV radiation. Sunlight was more optimum source of light as compared to tube light for the production of UV absorbing compounds. I effects of incubation time were mixed on different strains. Two weeks exposure were best time for isolate to producing absorbing compounds

Exposure of cyanobacterial cultures effectively induced the production of UV absorbing compounds in the isolate. *Synechocystis sp SCI* were the most responsive to UV light as their UV absorbing efficacy was maximum under such circumstances. Growth of cyanobacteria was highly affected by UV light. In case of *Synechocystis sp SCI* UV light was beneficial during exposure for 1, 3, 6 and 24 hours. Previously it was reported that UV exposure for different duration of time (10, 15, 30, 45 and 60 min) had negative influence on the growth of microalgae i.e. *Chlorella vulgaris* (Ganapathy *et al.*, 2017).

In case of *Synechocystis sp SCI*, maximum absorbance was at 258 nm which might indicate the presence of a quercetin compound with maximum absorbance at 254 nm (Campbell *et al.*, 1998). Similarly, absorbance at the wavelength of 268 nm, 280 nm, 234 nm and 238 nm might be due to the presence of MAA,s because this compound give single absorption band in the UV spectrum between 230 and 400 nm (Shourin *et al.*, 2014). Absorption of the wavelengths 308 nm, 336 nm and 350 nm indicated the production of scytonemin

which reportedly absorbs the wavelength of 252 nm to 350 nm (Sinha *et al.*, 1999). .

Cultures of isolated cyanobacteria grown for three weeks followed by exposure to UV were assayed for the presence of flavonoids. There was a gradual time dependent increase in the amount of flavonoids released into culture. Amount of total flavonoids in the culture of *Synechocystis sp SCI* was higher In UV exposed cultures, production of flavonoids in *Synechocystis sp SCI* was increased for 12 hours. Upon exposure to UV radiations, flavonoids production was significantly increased in *Kalanchoepinnata*. Influence of UV radiations on the production of flavonoids has been studied in plants but not in cyanobacteria. Flavonoids constitute an important component of anti-oxidant system in living organism which is active in soothing the damaging effects of a number of environmental stresses including UV radiations (Nascimento *et al.*, 2015). In addition, flavonoids may also be important in absorbing the UV radiations and hence safeguarding the delicate cell components from the damaging effect of harmful UV radiations (Ehling-Schulz *et al.*, 1999).

Cultures of isolated cyanobacteria grown for three weeks followed by exposure to UV were assayed for the presence of phenols. There was a gradual time dependent increase in the amount of phenols released into culture. Amount of total phenols in the culture *Synechocystis sp SCI* was higher. In UV exposed cultures the production of phenols in *Synechocystis sp SCI* were increased for 24 hours.

V. Conclusion

It is concluded that sun light is a good source for *Synechocystis sp SCI*. Most of the cyanobacterial isolates naturally produce and release UV-C absorbing compounds. However, exposure to UV light induces the production of UV-A and UV-B absorbing compounds. *Synechocystis sp SCI* produces more flavonoids in sun light and tube Production of phenols and flavonoids is greatly induced upon exposure of the isolated cyanobacteria to UV. Enhanced production of

phenols and flavonoids is beneficial for the protection of cyanobacteria against the damaging UV light.

REFERENCES

- [1] Barnett, J.Z., 2015. Effects of Light Quality and Light Quantity on the Growth Kinetics of a Louisiana Native Microalgal/Cyanobacterial Co-culture.
- [2] Barros, L., Baptista, P. and Ferreira, I.C., 2007. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food and chemical Toxicology*, 45(9), pp.1731-1737.
- [3] Becker, B., 2013. Snow ball earth and the split of Streptophyta and Chlorophyta. *Trends in Plant science*, 18(4), pp.180-183.
- [4] Becker, B., 2013. Snow ball earth and the split of Streptophyta and Chlorophyta. *Trends in Plant science*, 18(4), pp.180-183.
- [5] Boelen, P., Post, A.F., Veldhuis, M.J.W. and Buma, A.G.J., 2002. Diel patterns of UVBR-induced DNA damage in picoplankton size fractions from the Gulf of Aqaba, Red Sea. *Microbial Ecology*, 44(2), pp.164-174.
- [6] Carvalho, A.P., Silva, S.O., Baptista, J.M. and Malcata, F.X., 2011. Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Applied microbiology and biotechnology*, 89(5), pp.1275-1288. Carvalho, A.P., Silva, S.O., Baptista, J.M. and Malcata, F.X., 2011. Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Applied microbiology and biotechnology*, 89(5), pp.1275-1288.
- [7] Cockell, C.S. and Knowland, J., 1999. Ultraviolet radiation screening compounds. *Biological Reviews*, 74(3), pp.311-345.
- [8] Couradeau, E., Karaoz, U., Lim, H.C., Da Rocha, U.N., Northen, T., Brodie, E. and Garcia-Pichel, F., 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. *Nature communications*, 7, p.10373.
- [9] Dionisio-Sese, M.I., 2010. Aquatic microalgae as potential sources of UV-screening compounds. *Philipp. J. Sci*, 139, pp.5-16.
- [10] Ehling-Schulz, M. and Scherer, S., 1999. UV protection in cyanobacteria. *European Journal of Phycology*, 34(4), pp.329-338.
- [11] Ehling-Schulz, M., Bilger, W. and Scherer, S., 1997. UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. *Journal of Bacteriology*, 179(6), pp.1940-1945.
- [12] Ehling-Schulz, M., Bilger, W. and Scherer, S., 1997. UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. *Journal of Bacteriology*, 179(6), pp.1940-1945.
- [13] Ekebergh, A., Sandin, P. and Mårtensson, J., 2015. On the photostability of scytonemin analogues thereof and their monomeric counterparts. *Photochemical & Photobiological Sciences*, 14(12), pp.2179-2186.
- [14] El-Shehaw, R., Luomela, C., Ernst, A. and Bergman, B., 2003. Diurnal expression of heter and diazocyste development in the filamentous non-heterocystous cyanobacterium *Trichodesmium erythraeum*. *Microbiology*, 149(5), pp.1139-1146.
- [15] Gananath, K., Chidambaram, K., Janarthanan, R. and Ramasamy, R., 2017. Effect of UV-B radiation on growth, photosynthetic activity and metabolic activities of *Chlorella vulgaris*. *Journal of Microbiology and Biotechnology*, 6(2017), pp.53-60.
- [16] Garcia-Pichel, F., Belnan, J., Neuer, S. and Schanz, F., 2003. Estimates of global cyanobacterial biomass and its distribution. *Algalological Studies*, 109(1), pp.213-227.
- [17] Garcia-Pichel, F., Prufert-Behout, L. and Muvzer, G., 1996. Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. *Applied and Environmental Microbiology*, 62(9), pp.3284-3291.
- [18] Guilford, W.L., Schneider, D.M., Labovitz, J. and Onella, S.J., 1988. High resolution solid state ¹³C NMR spectroscopy of sporopollenins from different plant taxa. *Plant Physiology*, 86(1), pp.134-136.
- [19] Häder DP, Kumar HD, Smith RC, Worrest RC (2007). Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochem. Photobiol. Sci.* 6: 267-285.
- [20] He, Y.Y. and Häder, D.P., 2002. UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium *Anabaena* sp.: protective effects of ascorbic acid and N-acetyl-L-cysteine. *Journal of Photochemistry and Photobiology B: Biology*, 66(2), pp.115-124.
- [21] He, Y.Y., Klisch, M. and Häder, D.P., 2002. Adaptation of Cyanobacteria to UV-B Stress Correlated with Oxidative Stress and Oxidative Damage. *Photochemistry and photobiology*, 76(2), pp.188-196.
- [22] Karsten, U., Maier, J. and Garcia-Pichel, F., 1998. Seasonality in UV-absorbing compounds of cyanobacterial mat communities from an intertidal mangrove flat. *Aquatic Microbial Ecology*, 16(1), pp.37-44.
- [23] Liu, X.J. and Chen, F., 2003. Cell differentiation and colony alteration of an edible terrestrial cyanobacterium *Nostoc flagelliforme* in liquid suspension cultures. *Folia microbiologica*, 48(5), pp.619-626.
- [24] Lowe, N.J. and Friedlander, J., 1997. Sunscreens: rationale for use to reduce photodamage and phototoxicity. *Sunscreens: Development, Evaluation and Regulatory Aspects*, pp.35-58.
- [25] Maeda, H., Sakuragi, Y., Brvant, D.A. and DellaPenna, D., 2005. Tocopherols protect *Synechocystis* sp. strain PCC 6803 from lipid peroxidation. *Plant physiology*, 138(3), pp.1422-1435.
- [26] Matsumura, Y. and Ananthaswamy, H.N., 2004. Toxic effects of ultraviolet radiation on the skin. *Toxicology and applied pharmacology*, 195(3), pp.298-308.
- [27] Mushir, S., Deen, S. and Fatma, T., 2014. Screening of cyanobacterial strains for UV screening compound scytonemin-environmental perspectives. *Screening*, 3(2).
- [28] N Yadav, D., Barnawal, D., Maii, D. and Kalra, A., 2013. Exiguobacterium oxidotolerans, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennell under primary and secondary salt stress. *World Journal of Microbiology and Biotechnology*, 29(2), pp.379-387.
- [29] Nascimento, P.L., Nascimento, T.C., Ramos, N.S., Silva, G.R., Gomes, J.E.G., Falcão, R.E., Moreira, K.A., Porto, A.L. and Silva, T., 2014. Quantification, antioxidant and antimicrobial activity of phenolics isolated from different extracts of *Cassia frutescens* (Pimenta Malagueta). *Molecules*, 19(4), pp.5434-5447.
- [30] Paerl, H.W., Hall, N.S. and Calandrino, E.S., 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of the Total Environment*, 409(10), pp.1739-1745.
- [31] Pereira, A., Cao, Z., Murray, T.F. and Gerwick, W.H., 2009. Hoiamide, a sodium channel activator of unusual architecture from a consortium of two Papua New Guinea cyanobacteria. *Chemistry & biology*, 16(8), pp.893-906.
- [32] Pérez, G., Doldán, S., Borsani, O. and Irisarri, P., 2012. Differential response to moderate UV-B irradiation of two heterocystous cyanobacteria isolated from a temperate ricefield. *Advances in Microbiology*, 2(01), p.37.
- [33] Onesada, A., mouget, i.l. and vincent, w.f., 1995. Growth of antarctic cyanobacteria under ultraviolet radiation: uva counteracts uvb inhibition I.

- [34] Rao. P.L., Bhattacharva. R., Gunta. N., Parida. M., Bhaskar. A. And Dubev. R., 2002. Involvement of caspase and reactive oxygen species in cyanobacterial toxin anatoxin-a-induced cytotoxicity and apoptosis in rat thymocytes and Vero cells. *Archives of toxicology*, 76(4), pp.227-235.
- [35] Rastogi. R.P., Singh. S.P., Häder. D.P. and Sinha. R.P., 2010. Detection of reactive oxygen species (ROS) by the oxidant-sensing probe 2', 7'-dichlorodihydrofluorescein diacetate in the cyanobacterium *Anabaena variabilis* PCC: 7937. *Biochemical and biophysical research communications*, 397(3), pp.603-607.
- [36] Richa. K., Babbitt. C.W., Gaustad. G. And Wang. X., 2014. A future perspective on lithium-ion battery waste flows from electric vehicles. *Resources, Conservation and Recycling*, 83, pp.63-76.
- [37] Rozema. J., Biörn. L.O., Bormann. J.F., Gaberšček. A., Häder. D.P., Trošt. T., Germ. M., Klisch. M., Gröniger. A., Sinha. R.P. and Lebert. M., 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds. *Journal of Photochemistry and Photobiology B: Biology*, 66(1), pp.2-12.
- [38] Sahni. N., Singh. M.P., Bainai. U., Agarwal. A., Alimohammadian. H. And Sarkar. N., 2007. Ultrastructure of a Lower Eocene leaf surface impression in amber. *Vastan Lignite Mine, Gujarat. Jour. Paleont. Soc. India*, 52(1), pp.69-73.
- [39] Sargin. İ. and Arslan. G., 2015. Chitosan/sporonolinenin microcapsules: Preparation, characterisation and application in heavy metal removal. *International journal of biological macromolecules*, 75, pp.230-238.
- [40] Sargin. İ. and Arslan. G., 2016. Effect of glutaraldehyde cross-linking degree of chitosan/sporonolinenin microcapsules on removal of copper (II) from aqueous solution. *Desalination and Water Treatment*, 57(23), pp.10664-10676.
- [41] Shick. J.M., Lesser. M.P., Dunlan. W.C., Stochai. W.R., Chalker. B.F. and Won. J.W., 1995. Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acroporamicropthalma*. *Marine Biology*, 122(1), pp.41-51.
- [42] Sinha. R.P., 2015. Biochemical characterization of sunscreens/mycosporine-like amino acids from two Nostoc species inhabiting diverse habitats. *Protoplasma*, 252(1), pp.199-208.
- [43] Sinha. R.P., Klisch. M., Gröniger. A. And Häder. D.P., 1998. Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. *Journal of Photochemistry and Photobiology B: Biology*, 47(2-3), pp.83-94.
- [44] Sinha. R.P., Singh. S.P. and Häder. D.P., 2007. Database on mycosporines and mycosporine-like amino acids (maas) in fungi, cyanobacteria, macroalgae, phytoplankton and animals. *Journal of Photochemistry and Photobiology B: Biology*, 89(1), pp.29-35.
- [45] Sinha. Raieshwar; Klisch. M; Vaishampayan, Akhourj; Häder, Donat (1999-11-01). "Biochemical and spectroscopic characterization of the cyanobacterium *Lyngbya* sp. Inhabiting mango (*Mangifera indica*) trees: Presence of an ultraviolet-absorbing pigment, scytonemin". *Acta Protozoologica*. 38: 291–298.
- [46] SINHA. P., NAIK. S., AYYAGARI. A. & NAIK. S. R. (1998). The effect of *in vitro* bacterial association on virulence of *Entamoeba histolytica*. *Indian Journal of Medical Research* 105, 226–270
- [47] Soule. T., Palmer. K., Gao. O., Potrafka. R.M., Stout. V. and Garcia-Pichel. F., 2009. A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. *BMC genomics*, 10(1), p.336.
- [48] Stanier. R.Y., Kunisawa. R., Mandel. M. and Cohen-Bazire. G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriological reviews*, 35(2), p.171.
- [49] Steinmann. S.N. and Corminboeuf. C., 2011. Comprehensive benchmarking of a density-dependent dispersion correction. *Journal of Chemical Theory and Computation*, 7(11), pp.3567-3577.
- [50] Stevenson. C.S., Canner. E.A., Roshak. A.K., Marquez. B., Grace. K., Gerwick. W.H., Jacobs. R.S. and Marshall. L.A., 2002. Scytonemin—a marine natural product inhibitor of kinases key in hyperproliferative inflammatory diseases. *Inflammation Research*, 51(2), pp.112-114.
- [51] Takaichi. S. And Mochimaru. M., 2007. Carotenoids and carotenogenesis in cyanobacteria: unique ketocarotenoids and carotenoid glycosides. *Cellular and molecular life sciences*, 64(19-20), p.2607.
- [52] Tedetti. M. And Sennéré. R., 2006. Penetration of ultraviolet radiation in the marine environment. A review. *Photochemistry and photobiology*, 82(2), pp.389-397.
- [53] Urbach. E., Scanlan. D.J., Distel. D.L., Waterbury. J.B. and Chisholm. S.W., 1998. Rapid diversification of marine picoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria). *Journal of molecular evolution*, 46(2), pp.188-201.
- [54] Whitton. B.A. and Potts. M., 2012. Introduction to the cyanobacteria. In *Ecology of Cyanobacteria II* (pp. 1-13). Springer, Dordrecht.
- [55] Whitton. B.A., 2000. Soils and rice-fields. In *The ecology of cyanobacteria* (pp. 233-255). Springer, Dordrecht.
- [56] Xue. L., Zhang. Y., Zhang. T., An. L. And Wang. X., 2005. Effects of enhanced ultraviolet-B radiation on algae and cyanobacteria. *Critical reviews in microbiology*, 31(2), pp.79-89.
- [57] Zhang. Z., Wang. C.C., Zakaria. R. And Ying. J.Y., 1998. Role of particle size in nanocrystalline TiO₂-based photocatalysts. *The Journal of Physical Chemistry B*, 102(52), pp.10871-10878.
- [58] Zheng. W., Song. T., Bao. X., Bereman. B. And Rasmussen. U., 2002. High cyanobacterial diversity in coralloid roots of corals revealed by PCR fingerprinting. *FEMS microbiology ecology*, 40(3), pp.215-222.

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