

EFFECTS OF ULTRAVIOLET-B AND PHOTOSYNTHETICALLY ACTIVE RADIATIONS ON THE CYANOBACTERIUM *NOSTOC* SP. STRAIN HKAR-11

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Running Title: Effects of UV and PAR radiations on *Nostoc* sp.HKAR-11

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Abstract:

Continuous thinning of the ozone layer (stratospheric) due to anthropogenically released ozone depleting substance, such as chlorofluorocarbons, hydrochlorofluorocarbons, hydrofluorocarbons, perfluorocarbons and organobromides has resulted in enhanced influx of solar ultraviolet-B radiation (UV-B; 280-315 nm) on Earth's surface. Significant concerns have been pointed about the ill effects of UV-B (<1% of total irradiance) radiation on the usual biochemistry and physiology of sun-exposed organisms including cyanobacteria. The highly energetic UV-B has great potential to affect the metabolism of terrestrial and aquatic organisms, directly by effecting key cellular machinery such as proteins, DNA, lipids or induces indirect effects by aiding reactive oxygen species (ROS) generation and other free radicals as well. ROS-mediated protein modification, lipid-peroxidation, DNA damages such as formation of pyrimidine/ purine dimers and strand breaks, may result in ceasing of the vital functions of the cell. Moreover, ROS also inhibits photosynthesis by damaging the photosynthetic apparatus of cyanobacteria, therefore, any increase in this abiotic stresses would affect the survival of microbial cyanobacterial communities. In the present study effects of UV-B and photosynthetically active radiation (PAR) was studied on *Nostoc* sp. strain HKAR-11, a rice field isolate in terms of its survival, photosynthetic pigments content, total protein content and antioxidative enzymes activity.

Keywords: Cyanobacteria; Photosynthetically active radiation; Ultraviolet radiation; *Nostoc* sp. strain HKAR-11; Antioxidative enzymes

Introduction

Cyanobacteria are among the earliest inhabitants of Earth and their existence can be traced back to 3.8 billion years ago. They are an ancient group of photosynthetic prokaryotic organisms and are thought to be the first organisms to carry out oxygenic photosynthesis and are one of very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia through nitrification with the help of the enzyme "nitrogenase" (Vaishampayan et al., 2000, 2001; Pathak et al., 2018a; Pandey et

al., 2018). In the past few decades rapid industrialization has enhanced atmospheric pollutants (anthropogenically released) such as chlorocarbons, chlorofluorocarbons and organobromides that are responsible for the depletion of the UV-absorbing ozone layer in the stratosphere (Manney et al., 2011; Williamson et al., 2014). Different species of cyanobacteria illustrate a wide variation in tolerance to UV radiation and acquire a variety of defence strategies that aid their survival in environments having high UV-B fluxes (Pathak et al., 2019). A number of photoprotective mechanisms such as avoidance of brightly light habitats, production of UV-screening pigments/substances, quenching reactions for phototoxic products (reactive oxygen species; ROS), and repair of UV-induced damage have been observed in various cyanobacteria (Castenholz, 1996; Sinha and Häder, 2008; Singh et al., 2010; Pathak et al., 2018b, 2019; Rajneesh et al., 2018). The generation of ROS during photosynthesis is a normal phenomenon, however any stress condition provokes increased production of toxic oxygen derivatives (Pospíšil, 2009; Demidchik, 2010). This byproduct of biological redox reactions can inactivate enzymes, damage important cellular components and initiate lipid peroxidation, thereby producing lipid peroxyl radicals and lipid hydroperoxides (Halliwell and Gutteridge, 1989; Rajneesh et al., 2018). These in turn oxidize a range of cellular targets such as guanine bases in DNA, resulting into strand break (Britt, 1995; Cadet et al., 2012). During evolution of photosynthetic organisms several pathways aroused to prevent, or repair the negative effects of solar radiation by detoxification of ROS produced during UV stress, as well as the use of non-enzymatic antioxidants such as carotenoids, ascorbate (vitamin c) or α -tocopherol, and reduced glutathione and enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), or glutathione peroxidase (Castenholz and Garcia-Pichel, 2000; He and Häder, 2002; Pathak et al., 2018b, 2019; Rajneesh et al., 2018). UV-B radiation causes photobleaching of photosynthetic pigments, a decrease in phycocyanin/chlorophyll *a* ratio and disintegration of phycobilisome complex (Quesada and Vincent, 1997; Kannaujiya and Sinha, 2017; Kumar et al., 2018). In the present investigation response of a rice-field inhabitant cyanobacterium *Nostoc* sp. strain HKAR-11 on exposure to UV-B and photosynthetically active radiation (PAR) was studied in terms of its survival, photosynthetic pigments content, total protein content and antioxidative enzymes activity.

MATERIALS AND METHODS

IDENTIFICATION OF TEST ORGANISM

The cyanobacterium *Nostoc* sp. strain HKAR-11, a rice field isolate, was used to study the effects of UV-B and PAR. It is a member of Nostocales, characterized by frothy thallus, gelatinous, circinate trichome, absence of sheath, ellipsoidal cells and heterocyst us (**Fig. 1**) (Desikachary, 1959).

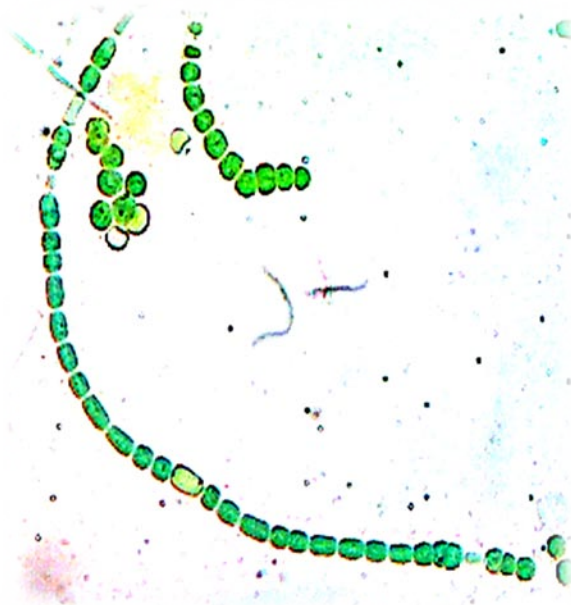


Fig.1. Photomicrograph showing the test organism *Nostoc* sp. strain HKAR-11.

CULTURE METHODS AND SOURCE OF UV IRRADIATION

Laboratory cultures of *Nostoc* sp. strain HKAR-11 were grown in BG-11 medium (Rippka et al., 1979) and incubated in a culture room at $28 \pm 2^\circ\text{C}$ and illuminated with fluorescent light of 12 W m^{-2} . Cultures were hand shaken five times daily. To avoid contamination, cultures were sub-cultured at regular intervals and all the experiments were performed during the log phase culture having an initial dry weight of $\sim 0.15 \text{ mg mL}^{-1}$ (7-8 days at 0.9 O. D. of total cell density at 750 nm). In a UV-chamber, the cyanobacterial samples were treated with artificial UV-B radiation in open glass Petri dishes (120 mm in diameter). Intensity of UV-B radiation was 1 W m^{-2} , which was maintained by adjusting the distance of the UV-B tubes in the chamber from the sample. Samples in the Petri dishes were covered with 295 nm cut-off filter foils (Ultraplan; Digefra, Munich, Germany). All experiments were carried out at constant temperature of $23 \pm 2^\circ\text{C}$ and to avoid heating effects during exposure, cultures were shaken at regular intervals. Untreated sample (0 h) served as control. Samples of irradiated cultures were drawn after at regular intervals and were harvested by centrifugation.

ESTIMATION OF CHLOROPHYLL AND TOTAL CAROTENE

Chlorophyll (Chl *a*) was estimated by the following method of Dere et al. (1998). Two mL cyanobacterial sample was centrifuged at 5,000 rpm for 10 min. Pellet was dissolved in 2 mL of methanol (100%) in a test tube. It was kept for 24 h at 4°C and centrifuged at 10,000 rpm for 5 min. Spectrum were taken at 250-700 nm against methanol as a blank using spectrophotometer. The cellular Chl *a* and total carotene content (using methanol as solvent) was calculated using following equations of Dere et al. (1998).

ESTIMATION OF PHYCOCYANIN

A known volume sample was taken and centrifuged at 10,000 x g for 10 min to obtain the pellet. Phycobilins were extracted from pellet using 0.05 M phosphate buffer by repeated freezing and thawing followed by sonication. The absorbance was read at 562, 615 and 652 nm against phosphate buffer as blank. Estimation of phycocyanin was done as per the method of Bennett and Bogorad (1973).

ESTIMATION OF PROTEIN

Protein was estimated by method of Lowry et al. (1951) using bovine serum albumin as standard.

DETERMINATION OF ANTIOXIDANT ENZYME ACTIVITY

For assaying antioxidant enzymes, cells from UV irradiated samples and control were harvested by centrifugation at 12,000 g for 15 min at room temperature. Preparation of cell extracts was done by sonicating the cells in 2 mL of extraction buffer under ice-cold conditions. Composition of the extraction buffer was 50 mM potassium phosphate buffer (pH 7.5), 1% (w/v) polyvinylpyrrolidone (PVP), 1mM ethylenediaminetetracetic acid (EDTA), 0.5% (w/v) Triton X-100. Supernatants were collected after centrifugating the homogenate at 10,000g for 10 min at 4°C, and used for assays of CAT and SOD. The method of Aebi (1984) was utilized for determining the CAT activity. APX activity was determined by measuring the decrease in absorbance of ascorbate oxidation to mono-dehydroascorbate at 290 nm for 1 min (Chen and Asada, 1989). Reaction mixture consisted of 650 mL (50 mM) phosphate buffer, 0.1 mM H₂O₂ (30 µL), 5 mM ascorbate and 200 µL of enzyme extract.

DETERMINATION OF PERCENT SURVIVAL

Aliquots (100 µL) were withdrawn at desired time intervals after UV-B and PAR exposure and plated on agar plates. Plates were kept in the dark for 48 h and thereafter transferred to light in the culture room. Colonies appearing after 15 days of growth were counted in a colony counter and percent survival was calculated.

STATISTICAL ANALYSES

All the experiments were repeated thrice with at least three replicates. One-way analyses of variance with multiple comparison modes were applied to evaluate the significance of data ($P \leq 0.05$).

RESULTS

EFFECT OF UV-B AND PAR ON CHL *a*, CAROTENE AND PROTEIN CONTENT

Prolonged exposure of UV-B radiation caused leaching of Chl *a* from cyanobacterial cells, finally leading to breakage of filaments. No significant decrement was observed in case of PAR. Chl *a* and total carotene content of cyanobacterial cells varied with the duration of UV-B and PAR exposure (**Fig. 2**). As duration of UV-B exposure increased, Chl *a* content of cyanobacterial cells decreased. However, in case of PAR Chl *a* content increased upto 6 h, thereafter it become constant (**Fig. 2A**). Total carotene content of cyanobacterial cells increased initially, which was followed by a decrease with increasing duration of different treatments (**Fig. 2B**). No significant change in total carotene content was observed in case of PAR. Protein content of cell increased upto 6 h of UV treatment thereafter it decreased on further exposure (**Fig. 3**). In case of PAR protein content increased upto 6h thereafter it became constant.

EFFECT OF UV-B AND PAR ON SURVIVAL AND PHYCOCYANIN CONTENT

We found that cell number decreased as the UV-B exposure time increased as compared to control, as after 24 h of UV-B treatment only 30 % viable cell was observed (**Fig. 4**). In PAR, 90 % cell remained viable after 24 h of exposure. More than 50% decrease was observed in phycocyanin content after 24 h of UV-B exposure as compared to control. Under PAR decrease was found upto 6 h of exposure thereafter it became constant (**Fig. 5**).

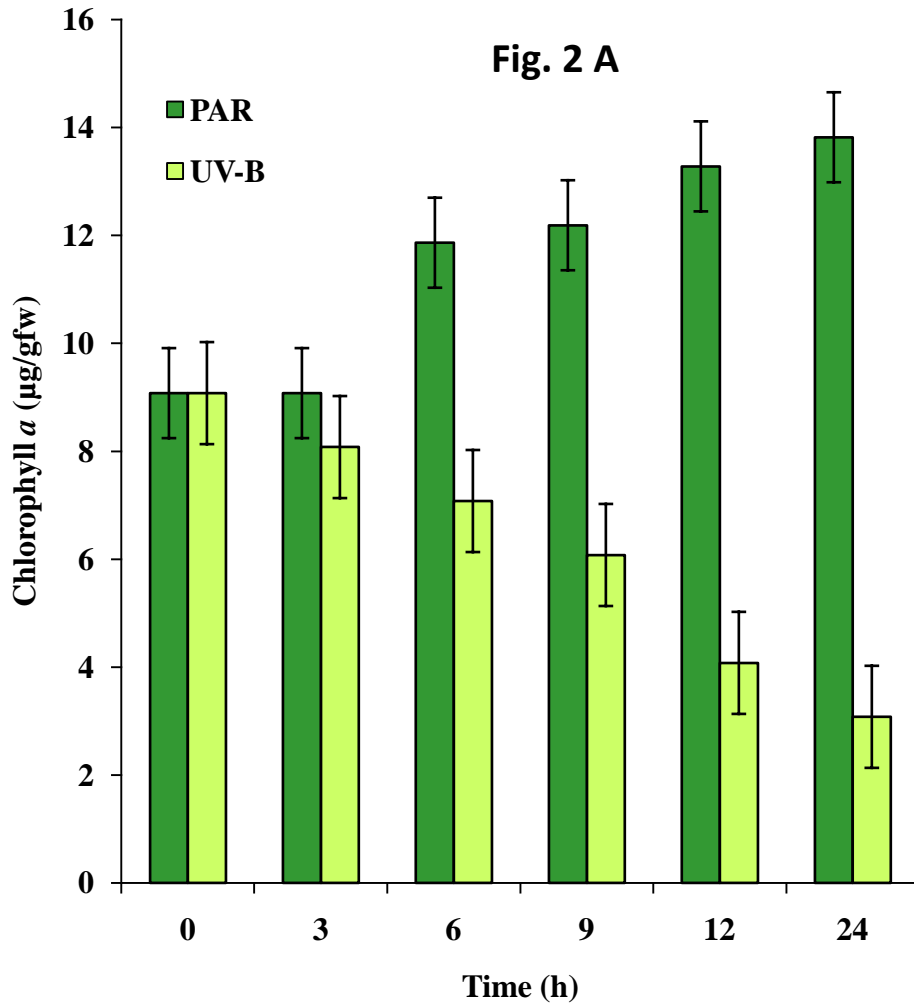


Fig.2. Effect of UV-B radiation and PAR on Chl *a* (A) and total carotene content (B) of *Nostoc* sp. strain HKAR-11 at 0, 3, 6, 9, 12 and 24 h of exposure. The error bar denotes standard deviations of means (n=3).

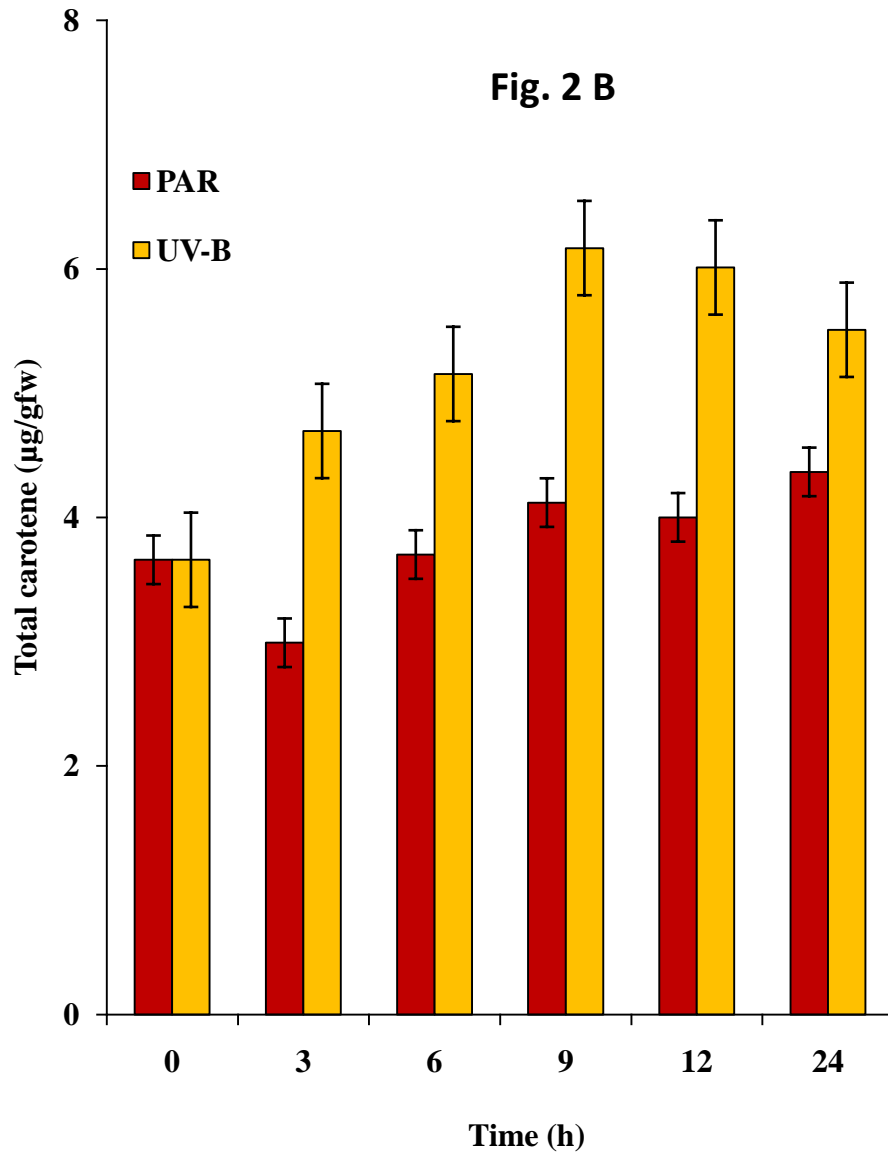


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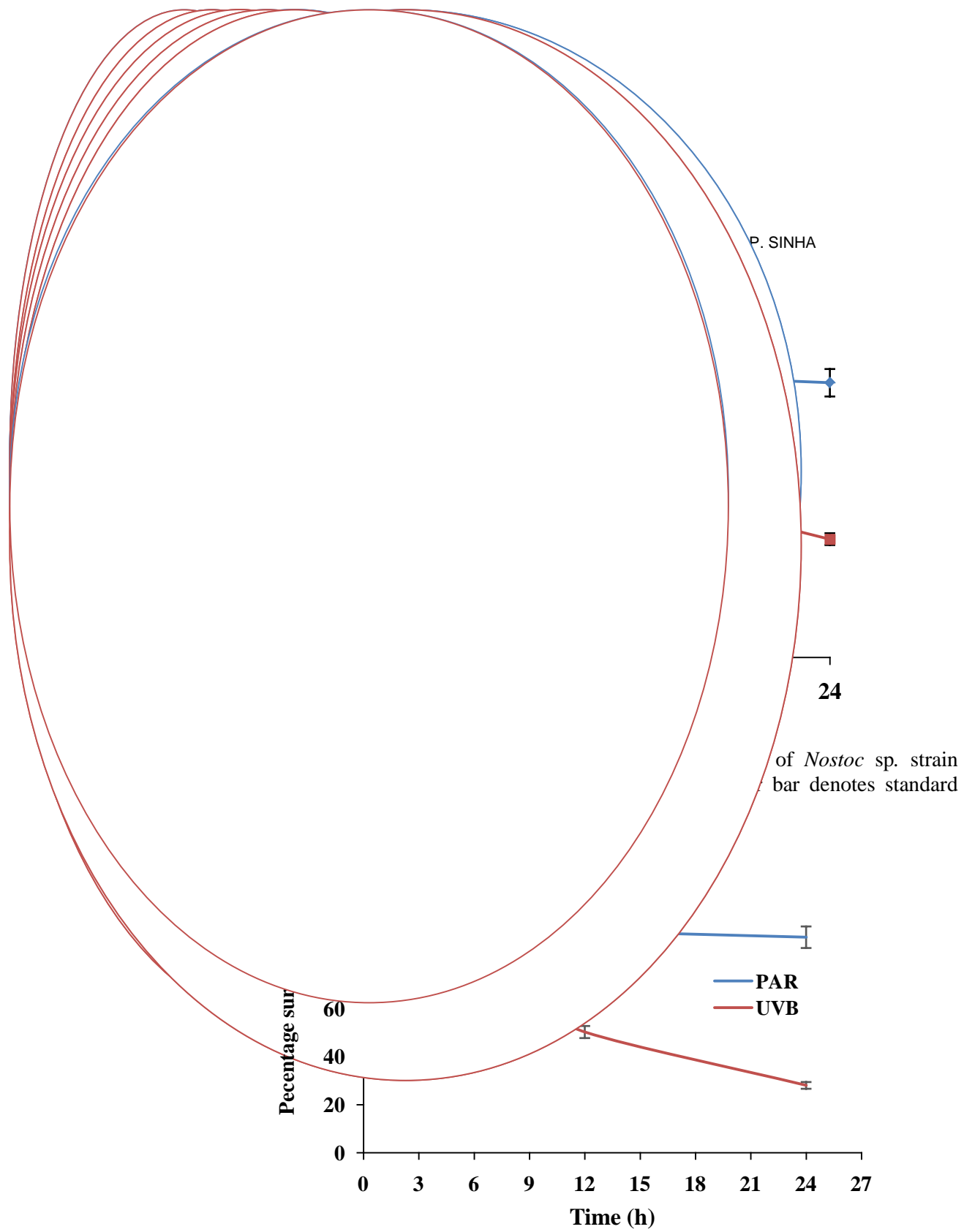


Fig.4. Effect of UV-B radiation and PAR on percentage survival of *Nostoc* sp. strain HKAR-11 at 0, 3, 6, 9, 12 and 24 h of exposure. The error bar denotes standard deviations of means (n=3).

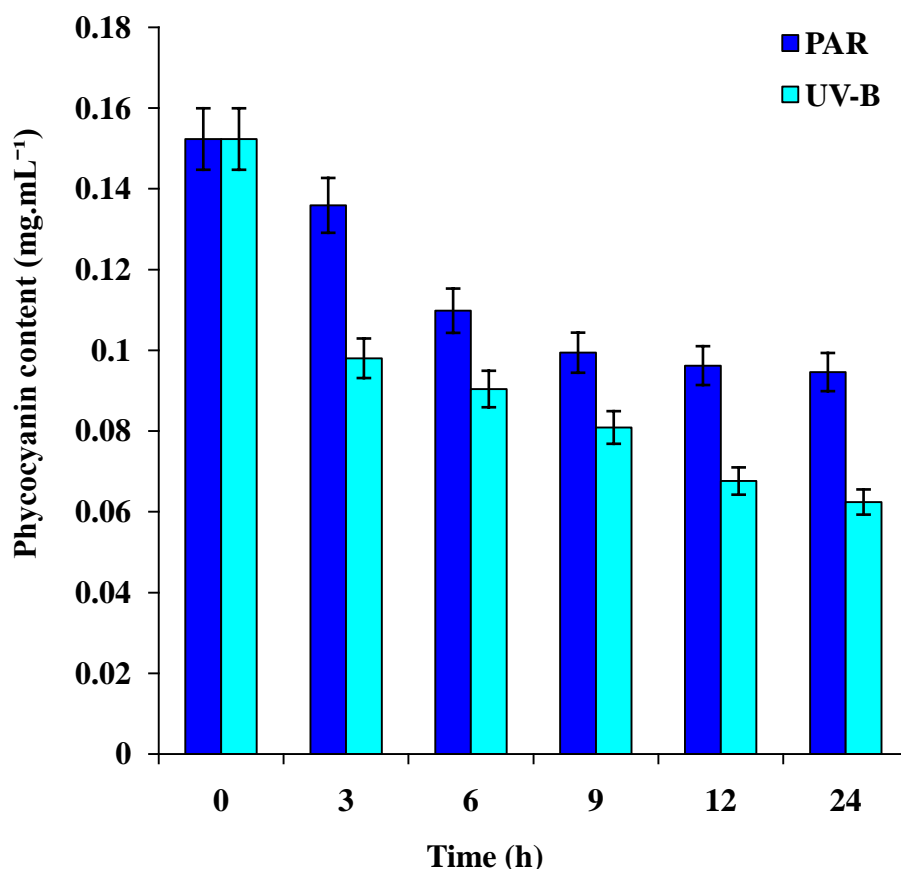


Fig.5. Effect of PAR and UV-B radiation on phycocyanin content of *Nostoc* sp. strain HKAR-11 at 0, 3, 6, 9, 12 and 24 h of exposure. The error bar denotes standard deviations of means (n=3).

EFFECT OF UV-B AND PAR ON ANTIOXIDATIVE ENZYMES ACTIVITY

UV-B stress induced significant change in the levels of the studied antioxidative enzymes. We observed 2.3 folds increase in CAT activity after 12 h of UV-B exposure but thereafter, its activity began to decline (**Fig. 6A**). Similar trend was observed in APX activity which reached a maximum of 0.361 U mg^{-1} of protein after 12 h of treatment, and then the activity declined after 12 h of exposure (**Fig. 6B**). No significant increase in CAT and APX activity was observed in PAR, only a slight increase was observed upto 6h of exposure thereafter it became constant.

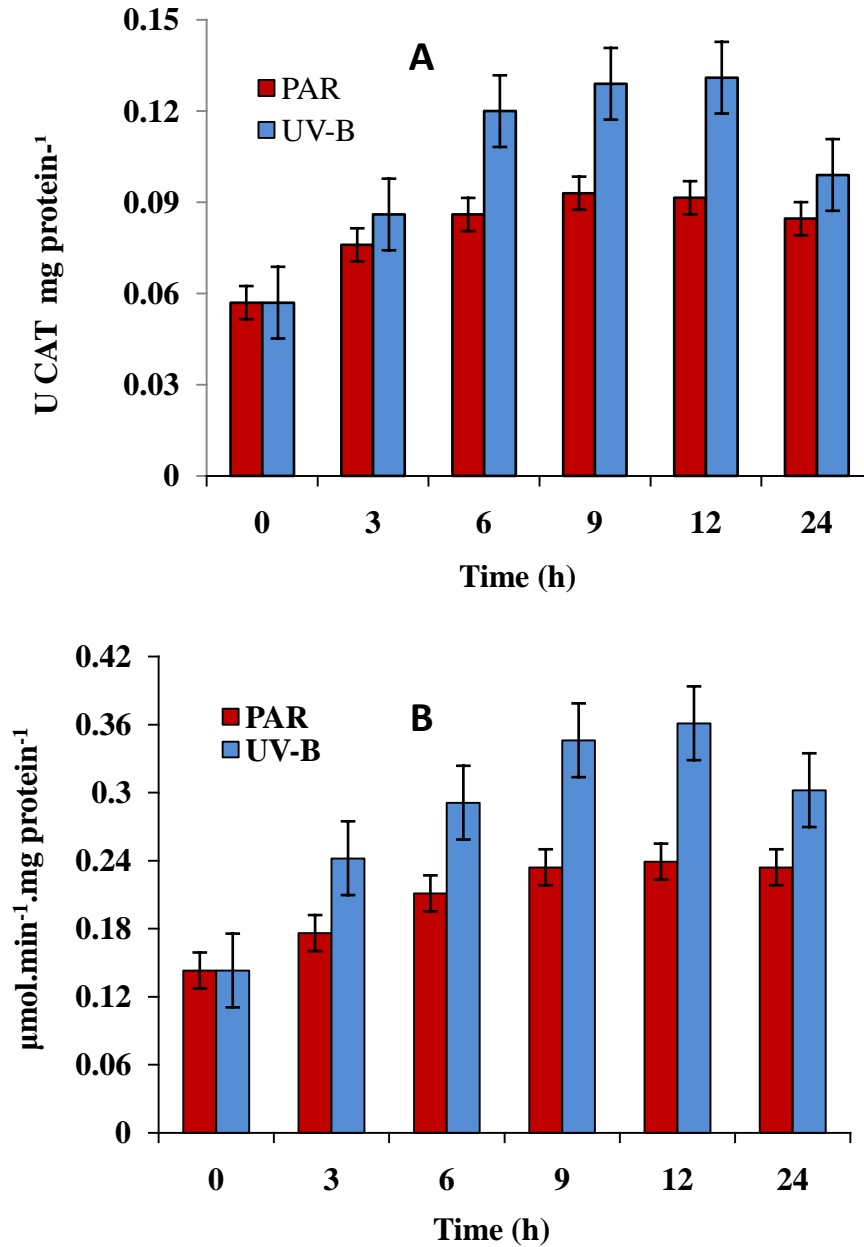


Fig.6. Activity of antioxidative enzymes in cultures exposed to UV-B radiation and PAR for varying time periods in *Nostoc* sp. strain HKAR-11. Catalase (A) Ascorbate peroxidase (B). The error bar denotes standard deviations of means (n=3).

DISCUSSION

Over the last decades numerous studies have been published on the effect of elevated UV radiation on terrestrial plants and cyanobacteria. In the present study response of *Nostoc* sp. strain HKAR-11 under UV-B stress was studied on certain biochemical processes. Estimation of percent survival under UV-B stress clearly indicated that *Nostoc* sp. strain HKAR-11 was susceptible to UV-B radiation as its percentage survival decreased to 30% after 24 h of treatment. Significant decrease in Chl *a* and phycocyanin was evident after 24 h of continuous UV-B exposure, although the effect was more pronounced on Chl *a*. Decrease in Chl *a* content may be attributed to photoreduction of protochlorophyllide to chlorophyllide under UV-B stress (Marwood and Greenberg, 1996). Additionally, bleaching of photosynthetic pigments by UV-B has been reported by few workers (Hargreaves et al., 2007, Rajneesh et al., 2018). Strong inhibition of phycocyanin by UV-B has also been reported and it seems that proteinaceous pigments may be the primary target of UV-B (Marwood and Greenberg, 1996; Lu and Vonshak, 2002, Kannaujiya and Sinha, 2017).

The increase in carotenoids may be to protect the organisms against the singlet oxygen species generated from oxidative damage by UV-B. Carotene can act as antioxidant to scavenge the free radicals. An overall increase in carotenoids in this study may suggest protection against the UV-B radiation by acting as antioxidant. Under UV-B stress enzymatic and non-enzymatic defense mechanisms are adopted by cyanobacteria (Singh et al., 2010). Induction of transcripts of antioxidative enzyme systems by UV-B radiation has been reported by several researchers (Jansen et al., 1998; Agrawal and Rathore, 2007; Singh et al., 2010; Richa and Sinha, 2015; Rajneesh et al., 2018). In present study multifold induction of CAT and APX activity was observed. Higher survival of *Nostoc* sp. strain HKAR-11 might be because of relatively higher levels of antioxidant enzyme activity such as SOD, APX even after 24 h of prolonged UV-B exposure. It has been reported that higher plants have suppressed levels of APX activity which in turn induces higher level of SOD, CAT and POD activity (Willekens et al., 1997; Richa and Sinha, 2015; Rajneesh et al., 2018).

Since we observed moderate levels of APX activity in *Nostoc* sp. strain HKAR-11, low levels of induction of CAT, activity seems justified in view of the above reports in higher plants. To a certain extent the induction of antioxidative enzymes such as CAT and APX is responsible for the survival even after 24 h of continuous UV-B stress in *Nostoc* sp. strain HKAR-11. However, the level of induction of the above enzymes may vary in different species under UV-B stress and that may govern the degree of survival. This explains the crucial role of antioxidant enzymes which maintain basal levels even after 24 h of UV-B exposure.

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