African Journal of Environmental Science and Technology Vol. 6(1), pp. 9-16, January 2012
Available online at http://www.academicjournals.org/AJEST
DOI: 10.5897/AJEST11.256
ISSN 1996-0786 ©2012 Academic Journals

Full Length Research Paper

Effects of ultraviolet-B (UV-B) radiation on two cryptogamic plants pigments growing at high altitude of central Himalayan region, India

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Accepted 18 December, 2011

Chlorofluorocarbons are mainly responsible for the depletion of stratospheric ozone layer which results in increase of UV-B radiation on earth's environment and causing adverse effects on flora. In the present study we have investigated the effect of ultraviolet-B (UV-B) radiation on two cryptogamic plants (*Xanthoria elegans* and *Bryum argenteum*) growing at high altitude of central Himalayan region of India. These plants were naturally receiving UV-B radiation and were analyzed for photosynthetic pigments, UV-B absorbing compounds and phenolics. In the field experiments, both of these plants contain higher amounts of UV-B absorbing compounds and phenolics and no major changes in total chlorophyll and carotenoid under UV-B exposed conditions were recorded. *B. argenteum* contains higher amounts of total chlorophyll, carotenoids, UV-B absorbing compounds and phenolics than the *X. elegans* at the duration of 120 h. The maximum average UV-B irradiance was 4.38 Minimal Erythemal Dose per hour (MED/ h) at the experimental site while minimum average UV-B irradiance was 1.72 MED/ h. The UV-B absorbing compounds and phenolics provide protection to these plants against UV-B radiation.

Key words: Total chlorophyll, high altitude, pigments, UV-B absorbing compounds, UV-B radiation.

INTRODUCTION

Depletion of the stratospheric ozone layer results in to increase in the ultraviolet-B (UV-B) radiation (280 to 320 nm) reaching the earth surface. Destruction of ozone layer is due to release of chlorofluorocarbons, resulting in stratospheric ozone thinning (Anderson et al., 1991; Schoeberl and Hartmann, 1991), consequently UV-B levels increases (McKenzie et al., 2003). The high intensity of UV-B radiation on earth's surface causes adverse effects on flora. The potential effects of UV-B radiation on phototrophic organisms may be grouped into three categories: (a) changes in photosynthesis and growth (Xiong and Day, 2001), (b) increased investment in UV-B absorbing or screening compounds (Cockell and Knowland, 1999; Searles et al., 2001) and (c) DNA damage, repair and photoreactivation (Lud et al., 2001a). UV-B radiation varies naturally with the latitude, season and depends on vegetation canopy, clouds etc., (Aphalo, 2003). According to Madronich et al. (1995) at high latitudes the relative ozone depletion is higher. Many of the studies conducted with vascular plants and bryophytes reveals that the UV-B effects were often highly variable, and depends on the species tolerance and UV-B doses. The naturally induced UV-B affects plant growth, morphology, secondary metabolism and photosynthesis (Allen et al., 1998; Searles et al., 2001; Pancotto et al., 2003). Plants are able to deal with UV-B induced stress because of UV-B absorbing compounds which are widespread and are found in lower to higher plants, including aquatic to terrestrial life forms (Rozema et al., 2002). One of the many roles of UV-B absorbing compounds and phenolics appears to be the protection of organisms from harmful effects of UV-B radiation by means of their direct absorption of 280 nm to 320 nm wavelengths. The phenolics were also protecting the plants from exposure of UV-B radiation, it may contribute

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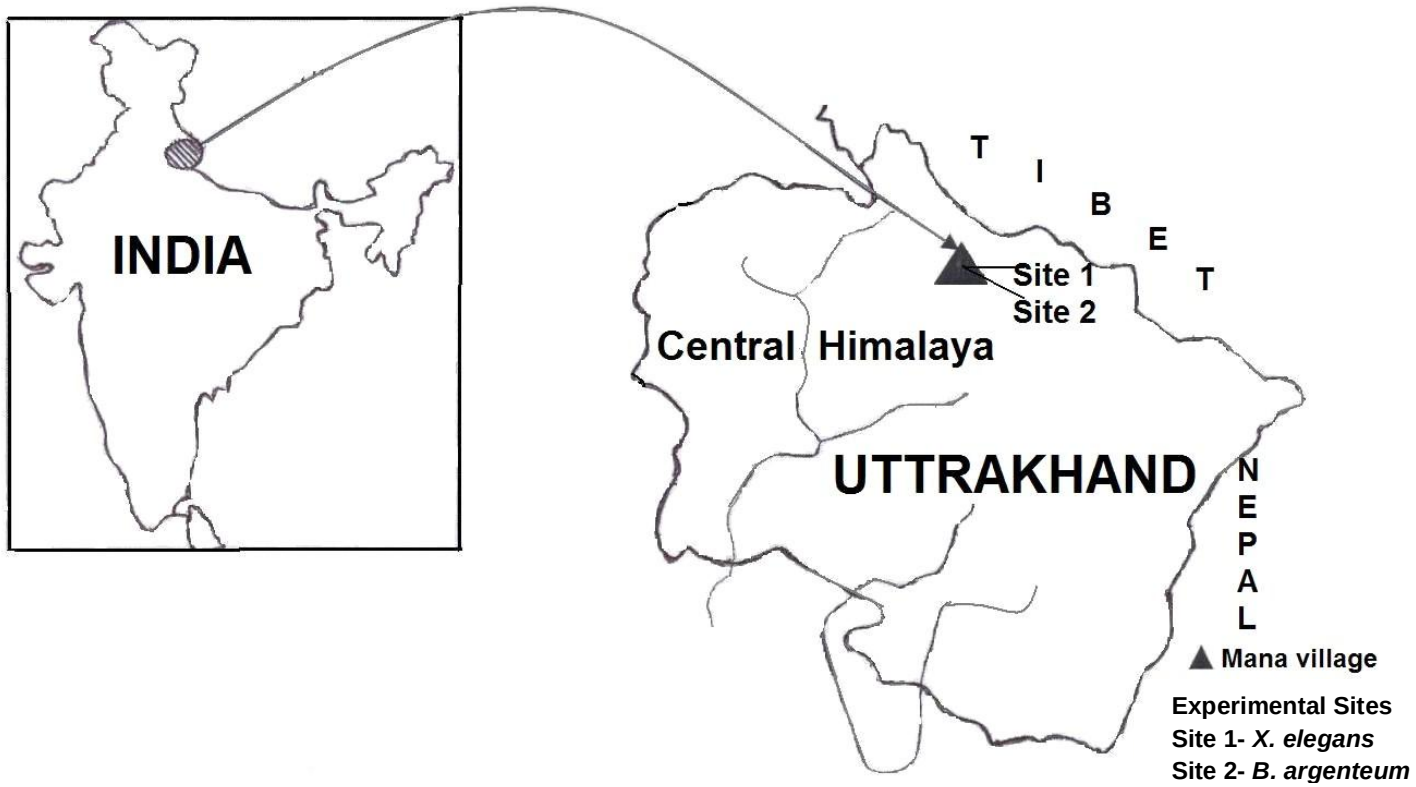

Figure 1. Map indicating selected sites for field experiments near Mana village, district Chamoli, Uttrakhand, India (www.mapsofindia.com).

to the decrease in active oxygen species by acting as antioxidants (Husain et al., 1987; Markham et al., 1998; Ryan et al., 2002).

In the present study, we have measured the UV-B radiation and their effects on the pigments of two cryptogamic plants (*Xanthoria elegans* and *Bryum argenteum*) growing at high altitude of central Himalayan region (near Mana village, district Chamoli of Uttrakhand), India.

MATERIALS AND METHODS

Site selection

For the UV-B measurements, the sites near Mana village (3219 m, altitude) of district Chamoli, Uttrakhand, central Himalayan region of India were selected. For the UV-B filter frame studies, with *X. elegans*, site 1 was selected at Bheempul (30°44'577N; 70°29'628E) of Mana village, and for the *B. argenteum*, site 2 (30°44'540N; 70°29'598E) was selected at the down side of the Bheempul (Figure 1).

Selection of plant species

Two plant species, lichen (*X. elegans*) and moss (*B. argenteum*) were selected and both were growing naturally on the mountains with other cryptogamic vegetation (Figure 2a and b). We have selected these plants for the field experiments because of their uniform growth, availability and UV-B filter frames can be placed over these plants.

UV-B filter frames

The UV-B filter frames were made up of iron stands which were covered by plexiglas acrylic sheet, so that plants will get PAR (photosyntheticaly active radiation) but not UV-B radiation. The acrylic sheet (3 mm thick and 30.5 cm squire) absorbs about 98% of total UV-B radiation. Iron frames having holes for gaseous exchange and the other environmental factors were same for both the conditions. At five sites, the UV filters frames (30.5 cm length × 30.5 cm width × 30.5 cm height) were placed over the selected plant species to develop UV-B unexposed conditions.

Analysis of pigments

All the analysis performed with two different experimental set up (i) UV-B exposed (plants without UV-B filter frame) and (ii) UV-B unexposed (plants covered with UV-B filter frame) conditions. Plant samples were harvested after 24, 48, 72, 96 and 120 h and washed with doubled distilled water, blotted dry on Whatman filter paper No.1 and their weight recorded. For the estimation of both pigments following standard methodologies used.

Analysis of photosynthetic pigments (total chlorophyll and carotenoids)

The plant samples were crushed with 80% acetone, maintained at 4°C and centrifuged at 10,000 rpm for 15 min under refrigerated centrifuge at 4°C temperature. The centrifuged samples were

Figure 2. Naturally growing plants of (a) *Bryum argenteum* and (b) *Xanthoria elegans* under UV-B exposed conditions.

filtered by Whatman filter paper No. 1 and the supernatant was collected. The absorbance of supernatants was recorded at 663, 645, 480 and 510 nm by using UV-VIS spectrophotometer 117. The chlorophyll content was calculated from absorbance values at 663 and 645 nm (Arnon, 1949) and the carotenoid content from absorbance values at 480 and 510 nm (Parsons et al., 1984).

Estimation of UV-B absorbing compounds

For the estimation of UV-B absorbing compounds, methodology of Ruhland and Day (2001) was used. The plant samples were placed in 25 ml Erlenmeyer flasks containing 5 ml of acidified methanol (MeOH:HCl:H_2O, 90:1:1 v/v). The supernatants were heated (60°C) and stirred for 10 min, cooled at room temperature for 15 min and filtered through 90 µm screens. Concentrations of soluble UV-B absorbing compounds were estimated by measuring absorbance at 300 nm with spectrophotometer (Systronics UV-VIS spectrophotometer 117).

Estimation of phenolics

For phenolics estimation methodology of Pirie and Mullins (1976) was used. A ten percent (w/v) homogenate prepared in methanolic HCl (50% methanol, 0.05% concentrated HCl, pH 3.5). The precipitate was allowed to settle for 15 h in the dark at 0-4°C and filtered through Whatman filter paper No.5. The absorbance of supernatant was recorded at 280 nm and gallic acid (Sigma chemicals, Germany) was used as standard.

Measurement of UV- B radiation

The UV-B radiation recorded by the UV- Biometer (Solar UV-Biometer, SOLAR LIGHT CO. 501, recorder S. No. 9343 and sensor S. No. 10402, U. K.), from 31st August 2008 to 5th September 2008 at the site of experiments during the clear sunny days.

Statistical analysis

The mean values along with standard error were calculated. The relative standard derivations of means were less than 5%. The student 't' test described by Fisher (1950) was employed to calculate the statistical significant values.

RESULTS

UV-B radiation

UV-B radiation (280 to 320 nm) were measured for the continuous 120 h at the selected sites and the average UV-B irradiances was 3.426 MED/h. The maximum average UV-B irradiance (4.38 MED/ h) was recorded at 72 h where as minimum was 1.72 MED/ h at 120 h (Figure 3). It was observed that the UV-B irradiance values were increasing from morning to noon and then decreased till evening.

Photosynthetic pigments

There was decrease ($p<0.02$) in total chlorophyll of UV-B exposed plants of *B. argenteum* at 96 h as compared to UV-B unexposed plants (Table 1) and at that time the UV-B irradiance was 3.67 MED/h. In UV-B exposed plants of *X. elegans*, the decrease ($p<0.05$) in total chlorophyll was found at 120 h as compared to UV-B unexposed plants (Table 1) and at that time the UV-B irradiance was 1.72 MED/h. The increase ($p<0.02$) in carotenoids of UV-B exposed plants of *B. argenteum* and *X. elegans* was recorded at 120 h as compared to UV-B unexposed plants (Table 2) and at that time the UV-B irradiance was 1.72 MED/h.

The significant increase ($p<0.02$) in UV-B absorbing compounds and phenolics of *X. elegans* were recorded at 120 h under the UV-B exposed conditions (Figures 4 and 5) and the values of UV-B irradiance was 1.72 MED/h. In *B. argenteum*, significant increase ($p<0.02$) in UV-B absorbing compounds and phenolics ($p<0.05$) were found at 120 h under UV-B exposed condition (Figures 6 and 7)

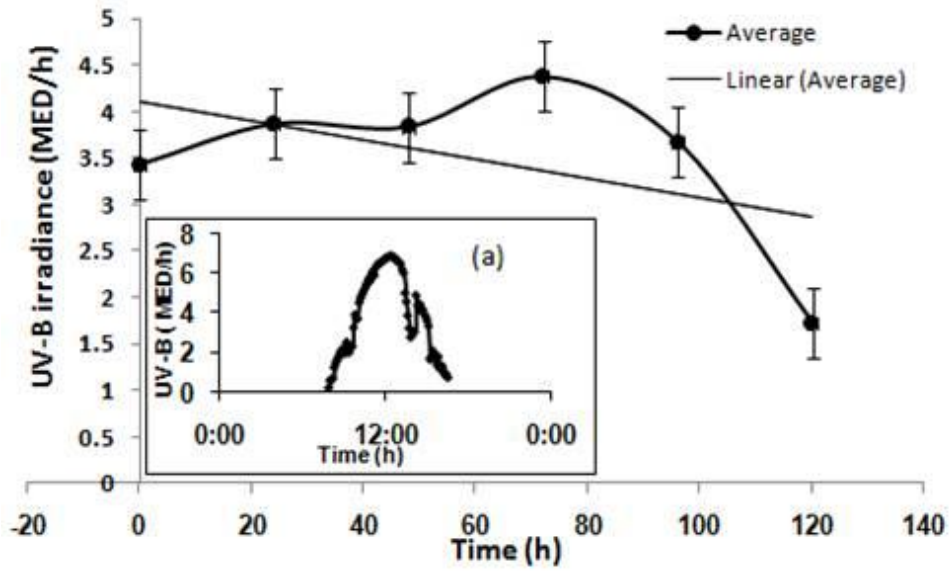

Figure 3. UV-B irradiance (MED/ h) from 0 h to 120 h. Inset: (a) Daily irradiance curve for one clear sunny day.

Table 1. Changes in total chlorophyll of *B. argenteum* and *X. elegans* (mg/ g fresh weight) in UV-B exposed and unexposed.

Plant species		Time (h)					
		0	24	48	72	96	120
B. argenteum	Unexposed	0.660±0.006	0.658±0.012	0.655±0.009	0.657±0.013	0.659±0.010	0.656±0.008
	Exposed	0.660±0.007	0.657±0.019	0.659±0.024	0.658±0.015	0.651±0.013	0.649±0.014
X. elegans	Unexposed	0.540±0.011	0.539±0.014	0.537±0.021	0.538±0.011	0.535±0.007	0.538±0.003
	Exposed	0.540±0.009	0.537±0.018	0.535±0.033	0.539±0.019	0.538±0.012	0.532±0.013

Above values are the mean±SE of three replicates.

Table 2. Changes in total carotenoid of *B. argenteum* and *X. elegans* (mg/ g fresh weight) in UV-B exposed and unexposed.

Plant species		Time (h)					
		0	24	48	72	96	120
B. argenteum	Unexposed	0.360±0.005	0.358±0.009	0.355±0.011	0.358±0.013	0.359±0.006	0.355±0.008
	Exposed	0.360±0.009	0.355±0.012	0.359±0.017	0.357±0.022	0.358±0.012	0.363±0.021
X. elegans	Unexposed	0.220±0.003	0.219±0.014	0.216±0.011	0.218±0.009	0.219±0.011	0.217±0.009
	Exposed	0.220±0.007	0.217±0.012	0.219±0.013	0.216±0.016	0.217±0.015	0.224±0.019

Above values are the mean±SE of three replicates.

and the UV-B irradiance was 1.72 MED/h. However, increase in the phenolics in *B. argenteum* is more or less same at 72, 96 and 120 h. The UV-B absorbing compounds and phenolics of both plants were positively associated with UV-B radiation exposure. In both the UV-B unexposed plants, there were no significant changes in UV-B absorbing compounds and phenolics during the course of the study.

DISCUSSION

The experimental evidences suggests that the ultraviolet radiation reaching on earth surface varies with altitude, atmospheric condition and types of instrument used for UV-B radiation measurement. Zaratti et al. (2003) reported that the erythemally weighted UV radiation increases with altitudes at an approximate rate of 7% per

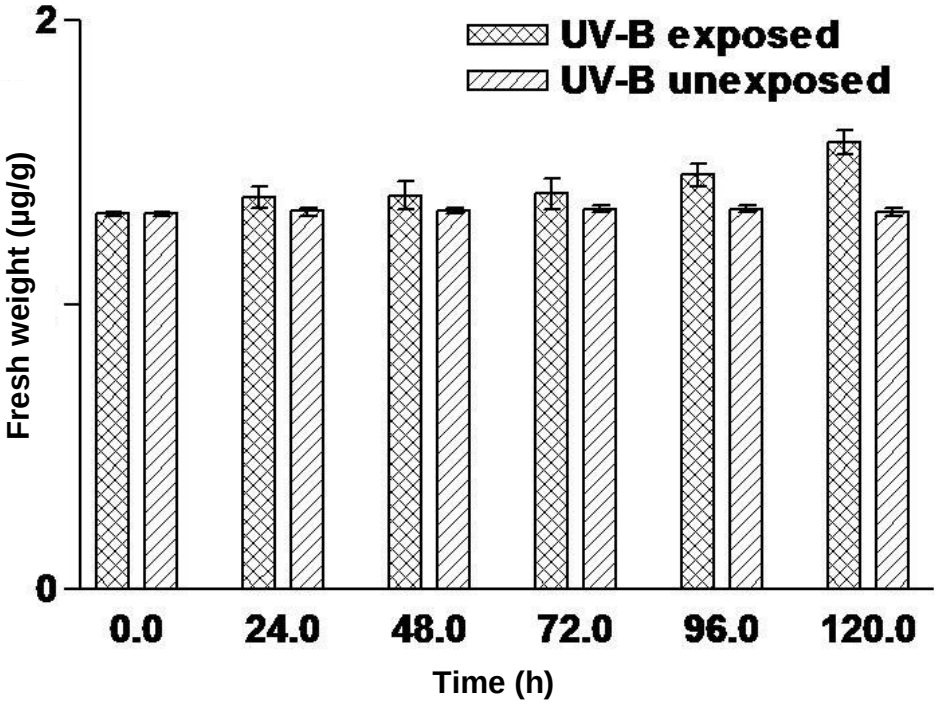

Figure 4. UV-B absorbing compounds in *X. elegans* under the UV-B exposed and unexposed conditions.

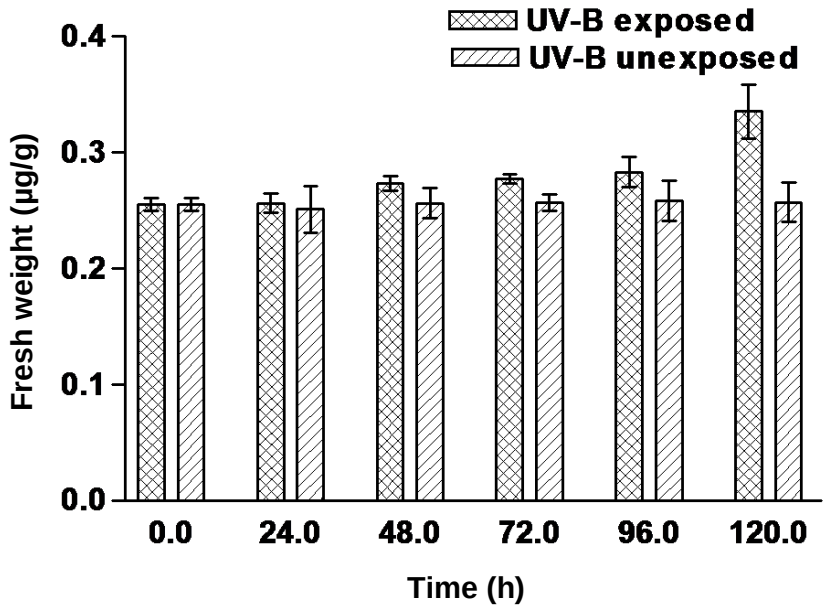

Figure 5. Phenolics in *X. elegans* under the UV-B exposed and unexposed conditions.

km. Mckenzie et al. (2003) also reported that the erythemally weighted UV irradiances increases by approximately 5 to 7% per km with the greatest increase occurring at solar zenith angle (SZA) ~ 60-70°.

In *B. argenteum* and *X. elegans*, total chlorophyll and carotenoids concentration showed no major changes at 24 h interval under UV-B exposed and unexposed conditions. Decrease in chlorophyll of *B. argenteum* and

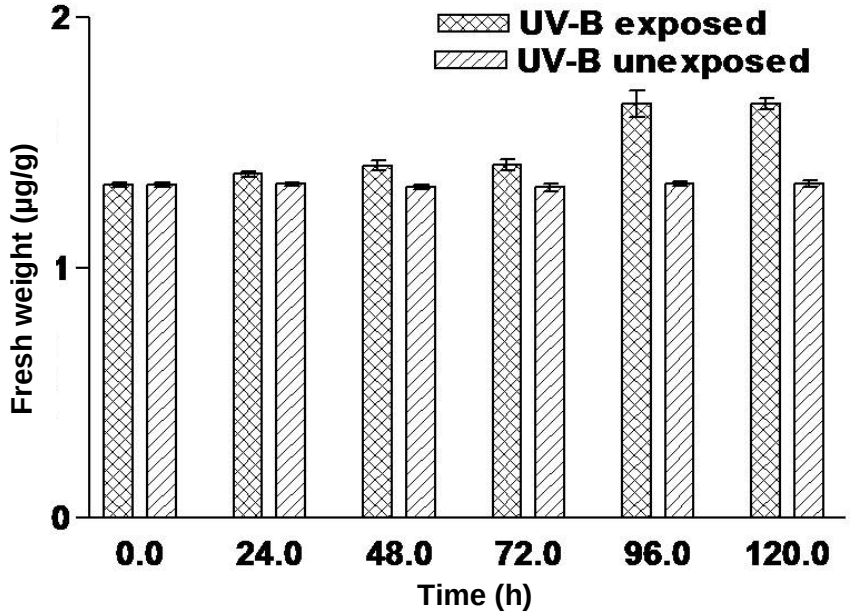

Figure 6. UV-B absorbing compounds in *B. argenteum* under the UV-B exposed and unexposed conditions.

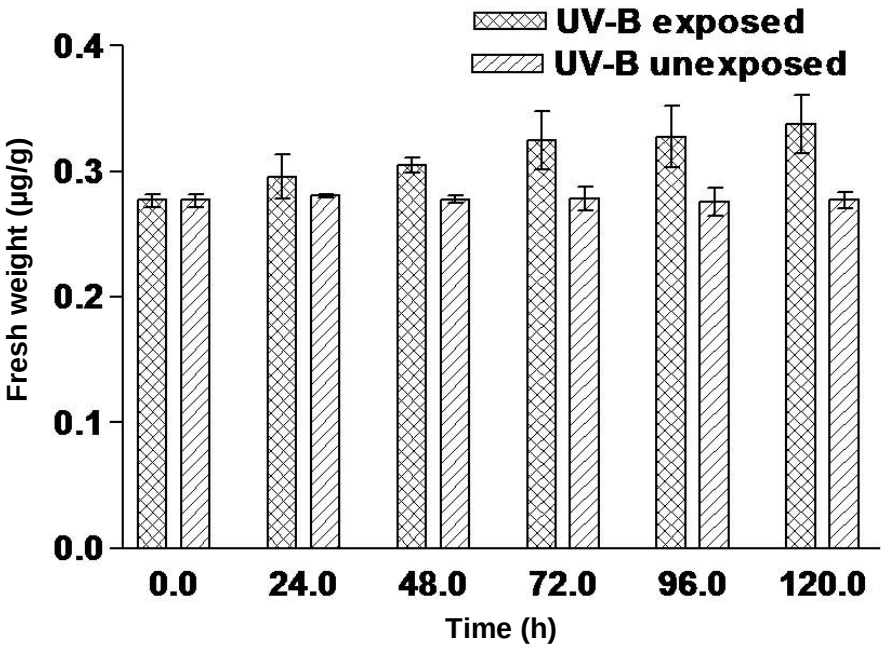

Figure 7. Phenolics in *B. argenteum* under the UV-B exposed and unexposed conditions.

X. elegans was found at 96 and 120 h respectively while increase in carotenoid of both the plants were found at 120 h. Newsham et al. (2002) conducted the similar UV related onsite study with two Antarctic plants (*Cephaloziella varians* and *Sanionia uncinata*) and found no change in photosynthetic pigments except increase in carotenoid. It was observed by Newsham et al. (2005) that the chlorophyll concentrations were reduced in sun exposed *C. varians* tissues. Robinson et al. (2005) found that the concentration of chlorophyll in *Grimmia antarctici* under near ambient UV radiation was lower and correspondingly high relative concentration of

carotenoids was recorded under reduced UV radiation. A similar study with lichen was conducted by Larsson et al. (2009) and documented no significant reduction in chlorophyll a and b on *Lobaria pulmonaria* and *Xanthoria aureola* at different UV-B levels (0, 0.1, 0.3 and 1.0 W m^{-2}) under the laboratory conditions. Lud et al. (2001b) did not found any differences in chlorophyll, carotenoid, UV-B absorbing compounds and photosystem II efficiency in *Turgidosculum complicatulum* exposed to various combinations of UV radiation and temperature. Day et al. (1999), Searles et al. (2001), Lud et al. (2002), and Newsham (2003) have found no effects of UV-B radiation on chlorophyll concentration of the plants.

We have found that UV-B absorbing compounds and phenolics in the exposed plants were increasing under the influence of UV-B radiation. Dunn (2000) reported that out of the three dominant mosses (*Bryum psudotriquetrum, Ceratodon purpureus* and *G. antarctici*) only *B. psudotriquetrum* produced UV-B absorbing pigments in response to increased UV-B radiation. Newsham et al. (2002) reported that the UV-B screening pigment concentrations of *C. varians* and *S. uncinata* were positively associated with daily doses of UV-B radiation in an *in situ* study conducted at Rothera point under 4 to 6 week. de la Rosa et al. (2001) reported that the concentrations of total phenolics were significantly increases by UV-B radiation. Dunn and Robinson (2006) reported that the higher concentration of UV-B absorbing compounds in two cosmopolitan moss with *B. psudotriquetrum* and *C. purpureus*, while *Schistidium antarctici* showing the lower concentration of UV-B absorbing compounds in response to UV-B radiation over the season (November 1999 - March 2000). Cockell and Knowland (1999) reported that the plants are accumulating the UV screening compounds in response to UV-B radiation stress. Rozema et al. (2002) and Singh et al. (2011) reported that the atronin, usnic acid, perlatolic acid and fumarphotocetraric acid were appeared to be constitutive in lichen, these all are UV-B absorbing compounds, takes a major part in lichens and are particularly induced by UV-B radiation. The UV-B absorbing compounds and phenolics are produced by the plants under the influence of UV-B radiation and thereby provides protection against the UV-B radiation. Therefore, in *B. argenteum* and *X. elegans* UV-B absorbing and phenolic might be responsible for providing protection against UV-B radiation.

Conclusion

Our study demonstrates the changes in pigments of two cryptogamic plants under UV-B exposed conditions at high altitude of central Himalayan region of India. *B. argenteum* and *X. elegans* were naturally exposed to UV-B radiation at study sites with a maximum average UV-B irradiance of 4.38 MED/ h and minimum average UV-B irradiance of 1.72 MED/ h during the study period. In both the UV-B exposed plants, UV-B absorbing compounds and phenolics were increasing during the study period. These findings suggest that the UV-B radiation induces synthesis of UV-B absorbing compounds and phenolics, therefore, these plants are able to deal with negative effects of UV-B radiation.

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