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UV-B Radiation and Its Differential Impact on Two Liverwort Species: An Eco-physiological Assessment

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Abstract:

The recent depletion of the stratospheric ozone layer—which normally filters out harmful ultraviolet-B (UV-B) radiation (280–315 nm)—has increased the need to understand how UV-B affects and is tolerated by photosynthetic organisms in different environments. In this study, two bryophyte species were exposed to elevated levels of UV-B radiation to assess its effects on growth and oxidative responses. The two species reacted differently, with Cythodium cavernarum showing greater sensitivity than Riccia discolor. A more pronounced decline in photosynthetic pigment content was observed in C. cavernarum. The effects on photosynthetic performance varied depending on both the UV-B dose and the species. Additionally, both enzymatic and non-enzymatic antioxidant systems were activated in a dose-dependent manner in both species; however, higher UV-B doses led to significant antioxidant loss and increased oxidative damage. Overall, the findings indicate that Cythodium cavernarum is more vulnerable to enhanced UV-B radiation compared to Riccia discolor.

Keywords: *UV-B, ROS, Antioxidant system*

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INTRODUCTION

UV-B radiation is mainly absorbed by biologically active macromolecules *viz*. proteins, nucleic acids and lipids, thus produce large photo-biological effects such as reduced photosynthesis, growth and biomass accumulation in plants [1,2]. Although considerable amount of data are available on physiological and biochemical responses of higher plants to elevated UV-B irradiation, very less attention has been paid to know the impact of UV-B on bryophytes, despite the fact that they are important contributors to the biomass in various ecosystems and good indicator of environmental stress [3]. Bryophytes exhibit simple morphological and anatomical features, however their adaptability to grow well in diverse habitats ranging from arctic, xeric to high altitudes may be one of

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the reasons for studying the UV-B induced responses. Their simple structure has led to suggestions that they might be exceptionally sensitive to enhanced UV-B irradiation [4]. Thus, they may exhibit interspecific as well as intraspecific variations with respect to UV-B sensitivity as reported in tropical legumes, soybean cultivars and cyanobacteria [5,6]. Such differences in UV-B sensitivity of plants could be associated with varied levels of active oxygen species (AOS) generation and defense strategies such as enhanced antioxidants and UV-B screening mechanisms.

The rise in generation of reactive oxygen species such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$), is known to be one of the early responses to UV-B irradiation leading to lipid peroxidation and membrane damage [7]. Even under optimal conditions, active oxygen species are generated as the byproducts of normal metabolism in different sub cellular compartments linked with photosynthetic and respiratory electron transport systems and many other biochemical pathways [8], but they are generated at high rates when plants are exposed to biotic and abiotic stresses. The excessive accumulation of AOS may peroxidise the important biomolecules, which causes further impairment of the photosynthetic electron transport and disruption of defense systems [9] resulting in oxidative damage at cellular levels. To mitigate and repair the damage initiated by AOS, plants have developed a complex antioxidant system. The enzymatic: superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR) etc, and non-enzymatic: proline, ascorbate, carotenoids, flavonoids etc, antioxidants [10,11] scavenge the oxidants ($O_2^{\bullet-}$), H_2O_2 and $^{\bullet}OH$) efficiently and thus regulate the extent of oxidative damage in cellular systems. Biosynthesis of these antioxidants and their cellular levels may directly be influenced by UV-B or indirectly through greater accumulation of AOS [9]. UV-B absorbing pigments like flavonoids offer protection in plant species by screening UV-B irradiation and thus, reduce the penetration of UV-B into the leaf interior [12, 13]. Plants exhibit great diversity, and different plant groups respond differently to the UV-B absorbing compounds, thus their concentration may vary from one species to another. Among bryophytes it has been shown that liverworts contain fairly good number of flavonoids, even more than mosses [14].

Recently, few studies have shown susceptibility of mosses and liverworts to UV-B [15,16, 17,18,19]. The study of Newsham [18] is of particular interest because in the two bryophyte species studied *Cephaloziella varians* and *Sanionia uncinata*, there was convincing evidence that the plants responded to increase or decrease of the incident UV-B in less than 24 h. Robinson *et al*. (2005) have also reported morphological variations and changes in pigment contents by present UV- levels in *Grimmia antarctici*, a plant with little UV-B absorbing compounds.

Bryophytes can serve as important tools for understanding UV-B induced responses as the majority lacks a cuticle, which can strongly absorb UV-B irradiation [20], however till date no initiative was taken to find out the susceptibility of two similar liverworts to UV-B at the biochemical level. Keeping above facts into consideration we have performed a comparative study on impact of UV-B on photosynthetic pigments and electron transport activities, levels of ROS, indices of oxidative damage and antioxidants status in two liverworts *Riccia discolor* L. *et* L. and C*yathodium cavernarum* Kunze, growing in same locality with different light requirements. *R. discolor* being xeric species mostly

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grows in moist and shady places and is sun acclimated, while C. *cavernarum* is shade acclimated species [21].

1. Materials and methods

1. Plant materials and growth conditions

Mature thalli of *Riccia discolor* L. and *Cyathodium cavernarum* Kunze were collected from Roxburgh Botanical Garden, University of Allahabad and nearby greenhouse of Chandrashekhar Azad Park, respectively during the month of September and October, 2005. Thalli were gently washed under tap water, followed by distilled water and placed in Petri dishes containing soil water extract-soaked filter papers. Open Petri dishes containing thalli were kept in growth chamber at 28 ± 2°C and illuminated 13 h a day with photon flux density of 50 μmol m^{-2} s^{-1} (PAR) provided by cool white fluorescent tube (Philips, 40 W).

2. UV-B exposure

R. discolor and *C. cavernarum* thalli were exposed to various doses of UV-B (20, 60 and 120 min of UV-B exposures, which correspond to 0.48, 1.44 and 2.88 K J m^{-2} , respectively), provided by UV-B fluorescent tube (Philips, TL 40 W/12, The Netherland) with its main output at 312 nm, together with 50 μ mol m^{-2} s^{-1} photosynthetic active radiation (PAR). The radiation was filtered through 0.127 mm cellulose diacetate (Johnston Industrial Plastics, Toronto, Canada) to remove all incidents UV-C (<280 nm). The irradiance was measured with the help of Power Meter (Spectra Physics, USA, model 407, A-2).

3. Photosynthetic pigments analysis

Chlorophylls and carotenoids from UV-B exposed and unexposed thalli were extracted with 80% acetone. The amount of chlorophyll was quantified by using the formula of Arnon (1949) and correction coefficient given by Porra (2002). Carotenoid contents were estimated by the method of Goodwin (1954).

4. Measurement of photosynthesis

Photosynthetic oxygen yield in thalli was measured with the help of O_2 electrode (Rank Brothers, UK) in the presence of 5 ml of 50 mM HEPES-NaOH buffer (pH 7.6) containing 20 mM $NaHCO_3$ as described by Kura-Hotta *et al.* (1987). Thalli were sliced into 1 mm wide strips in a Petri dish containing 10 ml of 0.5 mM $CaSO_4$. The sliced thalli were transferred into the vessel of oxygen electrode, and oxygen consumption (respiration) in darkness and evolution (photosynthesis) in light was estimated.

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For estimation of photosynthetic electron transport activities, chloroplasts were isolated from fresh thalli as described by Tripathy and Mohanty (1980) and then exposed to UV-B (0.4 Wm^{-2}) for 20, 40 and 60 min together with white light (20 µmol $m^{-2}s^{-1}$, PAR). The activity of PSII, whole chain electron transport and PSI was determined polarographically by Clark type oxygen electrode (Rank Brothers, UK). The rates of PSI (DCPIP/ASC→ MV) and whole chain (H_2O→ MV) in terms of O_2 consumption and PSII (H_2O→p-BQ) as O_2 evolution were estimated. Assay mixture consisted of 10 µM DCMU, 1 mM sodium ascorbate, 50 µM DCPIP, 50 µM sodium azide (NaN_3) and 50 µM methyl viologen (MV) for PSI; 50 µM NaN_3 and 50 µM MV for whole chain; 1 mM p-benzoquinone (p-BQ) for PSII measurements. In each case chloroplasts equivalent to 5 µg Chl ml^{-1} were suspended in the reaction mixture containing 0.5 M sucrose, 20 mM HEPES – NaOH buffer (pH 7.5), 10 mM NaCl and 10 mM $MgCl_2$. Spectrophotometric assay of PSII activity as DCPIP photoreduction in presence and absence of NH_2OH (5 mM) a artificial electron donor was monitored by measuring changes in absorption at 600 nm in reaction mixture containing 30 µM DCPIP.

5. Estimation of reactive oxygen species

For hydrogen peroxide (H_2O_2) estimation, 100 mg thalli from each set were crushed in 2.5 ml of 5% trichloroacetic acid and centrifuged at 400 g for 15 min, and total peroxide in supernatant was analyzed by the method of Sagisaka (1976). Superoxide radical ($O_2^{\bullet-}$) was measured as described by Elstner and Heupel (1976) by monitoring the nitrite formation from hydroxylammonium hydrochloride. A standard curve with NO_2^- was used to calculate the production rate of $O_2^{\bullet-}$ from chemical reaction of $O_2^{\bullet-}$ and hydroxylamine.

6. Indices of oxidative damage

Lipid peroxidation in each sample was determined by 2-thiobarbituric acid malondialdehyde (TBA-MDA) adduct formation as described by Heath and Packer (1968). The relative intactness of plasma membrane was measured as the leakage percentage of electrolytes as described by Gong *et al*. (1998). The UV-B exposed and unexposed thalli (300 mg) were washed thrice with deionized water and placed in test tubes containing 20 ml deionized water. The tubes were incubated in a water bath at 30°C for 2 h and the initial electrical conductivity (EC_1) of the medium was measured. The samples were boiled at 100°C for 15 min to release all electrolytes, cooled and final electrical conductivity (EC_2) was measured. Leakage percentage was calculated by using the formula [EC_1/ EC_2]100

7. Assay of the antioxidant enzymes

UV-B exposed and unexposed thalli were homogenized in 100 mM potassium phosphate buffer (pH 7.0) to extract catalase (CAT, EC 1.11.1.6) enzyme, while peroxidase (POD, EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1) were extracted in 150 mM potassium phosphate buffer (pH 6.1) and 100 mM EDTA – phosphate buffer (pH 7.8), respectively. The homogenates were filtered and centrifuged at 400 g for 15 min, and the supernatant was used for enzyme assay. CAT activity was

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determined polarographically with a Clark type oxygen electrode (Rank Brothers, U.K) as described by Teresa Milone *et al*. (2003). Oxygen produced by enzymatic reaction was calculated after correction for autoproduction of O_2 from H_2O_2. Temperature around the vessel was maintained at 25°C. One unit of CAT is the amount of enzyme producing 1μM O_2 min^{-1}. POD activity was determined specifically with guaicol at 470 nm following the method of Wu *et al*. (2003). The reaction mixture contained 50 mM potassium phosphate buffer (pH 6.1), 1% guaicol and 0.4% H_2O_2 in a 3 ml volume. The reaction was initiated by adding plant extract (150 μg protein) and an increase in the absorbance due to oxidation of guaicol (E = 25.5mM^{-1} cm^{-1}) was measured at 470 nm. One unit of POD is the amount of enzyme oxidising 1μM guaicol min^{-1}. SOD activity was assayed at 25°C according to the method of Giannopolitis and Ries (1977) using a reaction mixture (3 ml) containing 1.3 μM riboflavin, 13 mM methionine, 63 μM NBT, 0.05 M sodium carbonate (pH 10.2) and crude extract (100 μg protein). One unit of SOD is the amount of extract that gives half maximum inhibition.

8. Proline and ascorbate content

Proline content was determined by the method of Bates *et al*. (1973). UV-B exposed and unexposed thalli of both liverworts were crushed in 3% (w/v) aqueous sulfosalicylic acid, centrifuged. The supernatant was mixed with glacial acetic acid (3%, v/v) and acid ninhydrin (2.5%, w/v) and then kept for 1 h in water bath at 95°C. Samples were cooled, followed by extraction with 4 ml toluene. The absorbance of toluene layer was read at 520 nm and amount of proline was calculated from standard curve. Ascorbate content was determined according to Oser (1979) by extracting the thalli with 5%(w/v) sulfosalicylic acid. The supernatant was incubated at 60°C with 2%(w/v) of sodium molybdate, 0.15 N H_2SO_4 and 1.5 mM Na_2HPO_4, and absorbance was read at 660 nm.

9. UV-B absorbing pigments

UV-B absorbing pigments like flavonoids were extracted from UV-B exposed and unexposed thalli by keeping them for 24 h in acidified methanol (methanol: water: HCl :: 78: 20: 2)) at 40°C as described by Mirecki and Teramura [27]. The absorbance of supernatant was recorded at 320 nm, which is indicative of relative concentration of UV-B absorbing pigments.

3. Results

1. Photosynthetic pigments

UV-B exposures caused significant decrease in chlorophyll contents in both the liverworts (Table 1). The reduction in contents was dose as well as species dependent as *R. discolor* exhibited 4, 11 and 18% loss in chlorophyll content following 20, 60 and 120 min of UV-B exposures, respectively, while similar treatments resulted 6, 19 and 27% reduction in *C. cavernarum*. UV-B induced damaging effect on Chl *a* in *C. cavernarum* was found to be higher than Chl *b* showing low Chl *a* to Chl *b* ratio. Carotenoid contents showed differential response to UV-B exposures, as the level exhibited decreasing

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trend in *C. cavernarum*, while low UV-B dose (20 min) stimulated the carotenoid contents in *R. discolor*.

Table 1

Chlorophyll contents in *R. discolor* and *C. cavernarum* after 24 h of UV-B exposure

Photosynthetic pigment contents								
Treatment (UV-B exposure in min)	*R. discolor*				*C. cavernarum*			
	Chl *a* (mg g^{-1} Fw)	Chl *b* (mg g^{-1} Fw)	Total Chl (mg g^{-1} Fw)	Chl *a*/*b*	Chl *a* (mg g^{-1} Fw)	Chl *b* (mg g^{-1} Fw)	Total Chl (mg g^{-1} Fw)	Chl *a*/*b*
Control	0.24±0.01	0.20±0.01	0.44±0.01	1.13	0.18±0.01	0.16±.0.01	0.34±0.02	1.13
20	0.23±0.01 (-4)	0.19±0.01 (-5)	0.42±0.01 (-4)	1.16	0.17±0.01 (-6)	0.15±0.02 (-6)	0.32±0.01 (-6)	1.06
60	0.21±0.01 (-12)	0.18±0.01 (-10)	0.39±0.02 (-11)	1.14	0.14±0.01 (-22)	0.13±0.01 (-19)	0.27±0.02 (-19)	1.08
120	0.19±0.01 (-21)	0.17±0.02 (-15)	0.36±0.01 (-18)	1.07	0.13±0.01 (-28)	0.12±0.01 (-25)	0.25±0.03 (-28)	1.07

Values in parenthesis are per cent decrease (-) or increase (+) with reference to respective controls. mean ± S.E. (n = 3). Values with different superscripts are significantly ($P < 0.05$) different from each other (Duncan's multiple range test).

2. Photosynthetic measurements

Photosynthetic potential (total and net O_2 production) of both the liverworts under UV-B stress was examined and the results reveals that UV-B induced considerable reduction in photosynthetic oxygen yield in both the liverworts was more in *C. cavernarum* as there was 20 and 15% reduction in oxygen

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yield in *C. cavernarum* and *R. discolar*, respectively after 20 min of UV-B exposure, and the decreasing trend continued with increasing UV-B exposure. The rate of decrease in total oxygen yield was slightly less than net oxygen production, suggesting photosynthesis being more affected than respiratory process. The activities of PSII, whole chain electron transport and PSI in isolated chloroplasts directly exposed to UV-B showed dose dependent inhibition, however, the intensity of inhibition was stronger on PSII and whole chain than that of PSI. The extent of inhibition increased with increasing doses of UV-B. In *R. discolor*, 60 min of UV-B exposure caused 31% reduction in PS II activity, while *37*% loss was noticed in *C. cavernarum*. Similar UV-B treatment declined the whole chain electron transport by 37 and 40%, and PSI activity by 11 and 15% in *R. discolor* and *C. cavernarum*, respectively. The exogenous electron donor NH_2OH partially restored the PSII activity in both the liverworts, and the extent of restoration decreased with increasing UV-B exposure time. The extent of restoration in PSII activity was found to be more in *R. discolor* than *C. cavernarum.*

Table 2

Effect of UV-B exposures on photosynthetic electron transport activities in *R. discolor* and *C. cavernarum.*

Photosynthetic electron transport activities

[□mol O_2 evolved /consumed (mg Chl)$^{-1}h^{-1}$]

UV-B exposure (min)	*R. discolor*			*C. cavernarum*		
	PS I	PS II	Whole chain	PS I	PS II	Whole chain
Control	325±2	136±1	92±2	367±1	155±2	105±1
20	309±1	125±2	83±1	356±2	138±1	91±1
	(-5)	(-8)	(-10)	(-3)	(-11)	(-13)
40	302±1	109±2	70±3	345±2	116±3	73±2
	(-7)	(-20)	(-24)	(-6)	(-25)	(-30)
60	289±3	94±3	58±4	312±1	97±1	63±2
	(-11)	(-31)	(-37)	(-15)	(-37)	(-40)

Values in parenthesis are per cent decrease (-) or increase (+) with reference to respective controls. mean ± S.E. (n = 3). Values with different superscripts are significantly ($P < 0.05$) different from each other (Duncan's multiple range test**).**

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3. Active oxygen species production and oxidative damage

The effects of UV-B exposure on superoxide radical and H_2O_2 production in *R. discolor* and *C. cavernarum* thalli was investigated. The levels of AOS were significantly high in *C. cavernarum*, when exposed to UV-B and a dose dependant increase in superoxide radical and H_2O_2 content was noticed in both the liverworts. The H_2O_2 content almost doubled, although it was slightly more in *R. discolor* than *C. cavernarum*. Lipid peroxidation and percentage loss of electrolytes from membrane often reflects the oxidative stress encountered by the organisms. With increasing UV-B exposure time, significant increase in the MDA concentration and electrolyte leakage occurred in both the liverworts, however, the relative increase was more in *C. cavernarum* than *R. discolor*. Compared to control 120 min UV-B exposure caused 39% increase in MDA concentration in *C. cavernarum*, and only 25% in *R. discolor*. Similar UV-B treatment led to 35% electrolytes release in *R. discolor* and 45% in *C. cavernarum*.

Table 3

Effect of various exogenous electron donors on restoration of PS ll activity in isolated chloroplasts of two liverworts exposed to different doses UV-B.

	PS ll activity [µmol DCPIP reduced (mg Chl^{-1}) h^{-1}]			
UV-B exposure	*R. discolor*		*C. cavernarum*	
(min)	$-NH_2OH$	$+NH_2OH$	$-NH_2OH$	$+NH_2OH$
Control	160 ± 4	184 ± 4	181 ± 4	205 ± 4
20	145.6 ± 5 (9)	182.5 ± 6 (0.8)	159.3 ± 3 (12)	201.3 ± 5 (1.8)
40	129.6 ± 4 (19)	173.0 ± 5 (6)	130.3 ± 5 (28)	178.8 ± 7 (12.8)
60	110.4 ± 4 (31)	160.0 ± 5 (13)	105.0 ± 4 (42)	151.5 ± 6 (26.1)

Values in parenthesis are per cent decrease (-) or increase (+) with respect to respective controls. Mean ± S.E. (n = 3). Values with different superscripts are significantly ($P < 0.05$) different from each other (Duncan's multiple range test**).**

4. Antioxidative enzymes

The status of the antioxidative enzymes in UV-B treated and untreated thalli of both the liverworts was analyzed and results suggest that the activity of SOD, CAT and POD in the thalli varied significantly. The thalli of *R. discolor* exhibited appreciably high activities of SOD and POD, whereas

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CAT activity was considerably more in *C. cavernarum.* UV-B exposed thalli showed stimulated activity of SOD, CAT and POD, which showed declining trend with 120 min UV-B exposure. Interestingly the POD activity in *R. discolor* decreased considerably, which exhibited UV-B dose dependent inhibition. Beside this, in *R. discolor* thalli SOD activity increased even with 120 min of UV-B exposure.

5. non-enzymatic antioxidants (ascorbic acid and proline)

The ascorbic acid and proline contents were determined in UV-B exposed thalli of *R. discolor* and *C. cavernarum*. In control samples, *R. discolor* thalli contains appreciably more ascorbic acid and proline contents. In UV-B exposed thalli, the ascorbic acid content increased significantly in *R. discolor,* while *C. cavernarum* showed reverse trend. Interestingly *R. discolor,* which showed 13 and 17% increase in ascorbic acid content following 20 and 60 min of UV-B exposure, ascorbic acid content even than that of control following 120 min exposure. Proline content in *R. discolor* showed similar response as observed in ascorbic acid, while in *C. cavernarum* a progressive increase with increasing doses of UV-B was noticed.

6. UV-B absorbing pigments

Both the liverworts contained fairly good amount of UV-B absorbing pigments (flavonoids, OD at 320 nm), and the amount was almost double in *C. cavernarum* than that of *R. discolor*. The flavonoid contents in UV-B exposed thalli of both the liverworts increased with increasing exposure time, however 120 min UV-B exposure in *C. cavernarum* decreased the contents considerably, which was even less than in control thalli.

4. Discussion

In the present study, impact of different doses of ultraviolet-B radiation on certain physiological and biochemical activities of two thalloid liverworts namely *Riccia discolor* L. *et*. L. and *Cyathodium cavernarum* Kunze, the sun and shade acclimated species respectively were investigated. The study showed, UV-B induced significant inhibitions of photosynthetic pigments, including chlorophyll and carotenoids in both the liverworts with more damaging effect on *C. cavernarum* compared to *R. discolor,* suggesting higher sensitivity of the previous one. In general, UV-B radiation has been shown to destroy the photostability of chlorophylls directly (Strid and Porra, 1992), to inhibit the biosynthesis, and to accelerate the breakdown of pigments and their precurssors [12]. UV-induced alterations in chlorophyll contents of some bryophytes namely *Grimmia antarctici, Hyalocomium splendens and Polytrichum communae* has also been reported [17,19]. As for as carotenoids are concerned, beside protecting chlorophyll from photooxidative damage, they also serve as an antioxidant scavenging the free radicals and reactive oxygen species. They have a subsidiary role to UV-B screening pigments in protecting plants from UV-B radiations [22], thus loss in carotenoids as observed in the present study may lead to decreased UV-B filtrations and hence more damage. Studies have also shown that the

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ratio of Chl *a* to Chl *b* decreases [23], while total carotenoids to chlorophyll increases in UV-B exposed vascular plants and bryophytes [18,24].

C. cavernarum is more sensitive to UV-B treatments than *R. discolor* is also apparent from decreased photosynthetic potential and electron transport activities. The photosynthetic oxygen yield in intact system, and PS II and whole chain electron transport in isolated chloroplasts decreased significantly in both the liverworts with maximum reduction in *C. cavernarum* at high dose of UV-B. The loss in PSII activity might be due to inefficient energy transfer from light harvesting complex to the reaction center or inability of the reaction center to accept photons as a result of the structural alterations. The reaction center proteins i.e. D_1 and D_2, and oxygen evolving complex have been shown to be the main target of UV-B radiations (Renger *et al.*, 1989). Reduced photosynthetic activity in *Polytrichum cmmunae, Leucobryum undulatum and Pellia epiphylla* has been reported [16] suggesting the possible damage to PS II and overall state and functionality of the thylakoid membrane. The photosynthetic rate in UV-B exposed shade samples of *Fontinalis antipyretica* and liverwort *Jungermannia erectifolia* declined significantly more than the sun acclimated species [25]. Since NH_2OH, the exogenous electron donor donating the electrons very close to the reaction centre, failed to restore the PS II activity at high dose of UV-B, possibly the reaction center was damaged at the acceptor side, in addition to light harvesting system. The significant loss in PS II and whole chain electron transport activity in *C. cavernarum* revealed the greater sensitivity of shade samples towards UV-B. As observed in our study, PS I has been shown to be less sensitive than PS II against the stress.

Reduced photosynthetic activities and pigments may be resulted either due to direct effect of UV-B radiation or indirectly through increased accumulation of reactive oxygen species, as observed in the present study. The results showed heavy accumulation of ($O_2^{\cdot-}$) and H_2O_2 in both the liverworts. H_2O_2 produced as a result of dismutation of ($O_2^{\cdot-}$) and some other biochemical pathways is a potent inhibitor of photosynthesis and the destruction of H_2O_2 is vital to the functioning of chloroplasts [26]. The heavy accumulation of H_2O_2 in both the species with comparatively more increase in *R. discolor* than *C. cavernarum* suggests the importance of other factors either at the biochemical level or associated with membrane system in determining the sensitivity towards UV-B. However enhanced accumulation of reactive oxygen species ultimately caused the lipid peroxidation and thus increased electrolyte leakage from the cells [9] was observed. This also signifies the higher susceptibility of *C. cavernarum* as the MDA concentration, the end product of lipid peroxidation, and electrolyte leakage increased to a greater extent in this species. It has also been shown that lipid peroxidation coupled with reduction of carotenoid content may act synergically to decrease the energy transfer to the reaction center of PS II [26].

To counteract the toxicity of active oxygen species (AOS), plant cells contain a highly efficient antioxidant metabolites and enzymes that scavenge or prevent the accumulation of AOS thus, protecting cells from oxidative damage. Among enzymatic antioxidants, SOD catalyzes the dismutation of superoxide radical but merely transforms it into the more destructive H_2O_2, which is

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decomposed to water and molecular oxygen by catalases and peroxidases. The combined action of SOD and CAT is critical, mitigating the effects of oxidative stress. Both the species differed considerably in induction of antioxidant enzymes. Despite the higher SOD and POD activities, the CAT activity in control sample of *R. discolor* was less than *C. cavernarum.* Interestingly, the increase in SOD activities in *C. cavernarum* thalli were far more than in *R. discolor*, while CAT showed reverse trend. On the other hand, POD activity increased considerably in *C. cavernarum*, while in *R. discolor* it was strongly inhibited with increasing UV-B exposure. Thus, beside the amount and their stimulation or inhibition, the efficiency of antioxidants in various plant groups may also be the key determining factor in scavenging and accumulation of AOS.

Cellular low molecular weight non-enzymatic antioxidants also play an important role in inducing resistance to plants by protecting macromolecules against the damaging effects of free radicals and reactive oxygen species. The level of ascorbic acid was enhanced in *R. discolor*, whereas, a sharp decline was noticed in *C. cavernarum*. Enhanced level of ascorbic content in *Riccia* thalli possibly suggests its participation in detoxification of ROS, whereas the decline of ascorbic acid in *C. cavernarum* could be totally or partially due to consumption, while acting as antioxidant to limit lipid peroxidation.

Both the liverworts showed an enhanced accumulation of proline except in *R. discolor* exposed with 120 min UV-B. The high accumulation of proline might be attributed to the strategies adapted by the plants to cope with UV-B toxicity. Beside acting as osmoticum, proline also helps in scavenging of free radicals [27]. Apart from ascorbic acid and proline, flavonoids, play an important role in UV-B protection of plants as they show high absorption in the UV-B range [27]. Bryophytes are generally assumed to have low capacity to produce flavonoids [28], however in the present study two species contained fairly good amount of photoprotective and antioxidant flavonoids, although slightly more in *C. cavernarum* than *R. discolor*. The decreased flavonoid contents in 120 min UV-B exposed *C. cavernarum* thalli might be stress response affecting their synthesis. The synthesis of UV-B screening pigments is induced within several hours of exposure to UV-B radiation [29] owing to induction of genes encoding chalcone synthase, a key enzyme in the flavonoid biosynthesis pathway [29]. Considerable quantities of UV-B absorbing pigments have also been found in other bryophytes including *Cephaloziella varians, Saniona uncinata* and *Andraeaea regularis* [18], so it seems likely that the UV-B protection is not uncommon [19].

Conclusions

The present study demonstrates that ultraviolet-B (UV-B) radiation exerts significant adverse effects on the physiological and biochemical processes of the two thalloid liverworts, *Riccia discolor* and *Cyathodium cavernarum*, with *C. cavernarum*—a shade-acclimated species—being more sensitive than the sun-acclimated *R. discolor*. UV-B exposure led to a marked reduction in photosynthetic pigments, especially chlorophylls and carotenoids, impairing photosynthetic efficiency and electron transport activities, particularly in *C. cavernarum*. The observed decline in PSII activity, which could

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not be restored by exogenous electron donors, suggests damage to both the light-harvesting complex and the reaction center, underlining the vulnerability of shade-adapted species to UV-B stress.

The study also highlights the role of oxidative stress in mediating UV-B-induced damage, as evidenced by elevated levels of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage. Although both species activated antioxidant defense mechanisms, including enzymatic antioxidants (SOD, CAT, POD) and non-enzymatic antioxidants (ascorbic acid, proline, flavonoids), their efficacy and modulation differed. *R. discolor* showed a more coordinated antioxidant response, particularly higher ascorbate accumulation and sustained POD activity, potentially contributing to its greater tolerance. In contrast, *C. cavernarum* exhibited heightened ROS accumulation and less effective antioxidant buffering under prolonged UV-B exposure.

These findings underscore the differential sensitivity of liverwort species to UV-B radiation, shaped by their ecological adaptation, and emphasize the importance of antioxidant defense systems in mitigating UV-B-induced oxidative damage. The study contributes valuable insights into the UV-B stress physiology of early land plants and highlights potential traits linked to photoprotection and stress resilience in bryophytes.

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