

## **On the possible influence of short-term ultraviolet-B (285-325 nm) radiation on growth, pigment composition and photosynthetic activity in aquatic higher plant *Lemna gibba* L.**

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### **ABSTRACT**

*The impact of short-term UV-B radiation on growth, pigment composition and photosynthetic activities was studied in intact *Lemna gibba* L. fronds. A daily UV-B exposure for 30 min at 5 W.m<sup>-2</sup> resulted in significant changes on the morphology as well as physiological characteristics. Besides changes in biomass and relative growth rate, photosynthetic pigments such as Chl a, b and carotenoids decreased significantly. The level of non-photosynthetic pigments such as anthocyanin and flavonoids increased under UV-B treatment playing a defensive role. The photosynthetic activity measured in terms of O<sub>2</sub> evolution and Chl a fluorescence induction confirmed the vulnerability of aquatic species to UV-B radiation. Thus, the impact of short-term UV-B treatment was found to have pronounced effects in aquatic plants than that of terrestrial plants.*

**Key words:** Fluorescence, growth, impact, *Lemna*, UV-B.

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### **INTRODUCTION**

During the last 60 years, stratospheric ozone has decreased by about 5%, mainly due to the release of ozone-destroying anthropogenic pollutants such as chlorofluorocarbons resulting in higher levels of UV-B (280–320 nm) radiation at the Earth's surface (UNEP, 2002). Depletion of stratospheric ozone leads to enhanced level of incoming solar UV-B. Current global terrestrial UV-B radiation range between 2 and 12 kJm<sup>-2</sup> d<sup>-1</sup> on a given day with near equator and mid-latitudes receiving higher doses with an increase of 6–14% since 1980s. Solar UV-B flux is not uniform throughout the earth's surface but vary gradually from high to low latitude regions. The biological effects of enhanced solar UV-B include reduction in plant growth, photosynthetic activity and biomass. Several investigations of the effects of UV-B on plants have been carried out in response to concern over decreasing global stratospheric ozone and a concomitant increase in tropospheric UV-B, [1] [2]. Inhibitions of growth, photosynthesis and phytomass accumulation are some of the UV-sensitive basic responses. The effects of increased solar UV-B radiation on terrestrial and aquatic plants have been extensively reviewed [3] [4] [5]. Accumulation of proline and ABA was reported in UV-B irradiated wheat. [6]. This is the first report on short-term UV-B effects in an aquatic higher plant system. Although the UV-B effects in aquatic plants resemble that of terrestrial systems, the impact seems to be much higher in aquatic systems.

However, tropical plants [7] and temperate Brassicas [8] were shown to have increased susceptibility to photoinhibition including UV-B radiation. [9] have shown that visible light and UV-B radiation individually induce photodamage of PSII via two distinct mechanisms and that UV-B radiation can enhance repair of visible light

damage in plants, albeit only at low light intensities. Although multiple target sites exist for UV-B, PSII was shown to be the primary target [10].

## MATERIAL AND METHODS

**Plants:** *Lemna gibba* L. fronds were maintained in plastic troughs of 30 cm diameter and 20 cm depth with filtered tap water having an electric conductivity of  $0.8 \text{ mmhos cm}^{-1}$ . A mixture of 200 g wet land clay soil, 5 mg of superphosphate, and 50 g of fresh cow dung were placed in each trough to which  $4,000 \text{ cm}^3$  of water was added to make slurry. The slurry was allowed to settle and to each trough 1 g of juvenile fronds of *Lemna* was added. The water level was maintained at 10 cm above the slurry throughout the study period. In addition, fur a don ( $50 \text{ g.m}^{-3}$ ) was sprayed to control pests. The troughs were placed in partial sunlight in the shade house with the day/night temperature of  $32/26 \text{ }^\circ\text{C}$ .

### 2.1 UV-B treatment

*Lemna* fronds received UV-B (285-325 nm) radiation for 30 min per day during 12:00 and 12:30 h from a 20 W UV-B lamp (Gloelampenfabriken, Holland) placed at a distance of 30 cm above the troughs. UV-B radiance was monitored using an IL 700A radiometer equipped with a broad band photodiode, type SEE 400 W (International Lights Inc., USA). Visible radiation was measured using a LI 188 quantum/radiometer (Li-Cor Inc., USA).

### 2.2 Pigment analysis

Fresh *Lemna* fronds of 100 mg were weighed and homogenized in 100% acetone using a mortar and pestle. The homogenate was filtered and spun at 4000 rpm for 5 minutes at  $4 \text{ }^\circ\text{C}$ . The extraction was repeated with 100% acetone until a pale-yellow or white color pellet is obtained. The supernatants were pooled after each centrifugation discarding the pellet. The supernatant was used to measure the absorbance at 662 nm, 645 nm and 470 nm for Chlorophyll *a*, *b* and carotenoids respectively. Chl *a*, Chl *b*, total Chlorophyll and carotenoids were calculated using the formulae of [11].

*Lemna* fronds were soaked in  $10 \text{ cm}^3$  of acidified methanol (methanol:water:HCl; 80:20:1 v/v/v) and left overnight at  $20 \text{ }^\circ\text{C}$ . The absorbance of the clear extract was measured at 315 nm to quantify the flavonoid content. For anthocyanin estimation, the fronds were crushed in the above solution and the extract was cleared and absorbance measured at 530 and 657 nm.

### 2.3 Estimation of Photosynthetic activity ( $\text{O}_2$ evolution)

The rate of PSII mediated  $\text{O}_2$  evolution was continuously monitored at  $25^\circ\text{C}$  with intact fronds using a leaf disc oxygen electrode (Hansatech, U.K.). Prior to measurement, the capillary mat was wetted with 1 M  $\text{HCO}_3$  buffer to facilitate rapid gas exchange. *Lemna* fronds of  $2 \text{ mm}^2$  were placed in the electrode chamber and exposed to saturating light intensity of  $600 \mu\text{mol m}^{-2}$  using a photo phone slide projector as light source. The measurement procedure was same as that of [12].

### 2.4 Chl *a* fluorescence induction kinetics

#### Fast and slow Fluorescence transients

In vivo Chl *a* fluorescence transients were followed in intact fronds after excitation with broad band blue light (400-620 nm, Corning, CS4-96) at a photon flux density of  $100 \text{ W.m}^{-2}$ . The photomultiplier (Hamamatsu R376) placed at  $90 \text{ }^\circ\text{C}$  to the excitation beam was protected by an interference filter ( $\lambda \text{ max } 690 \text{ nm}$ , half band width 12 nm, Schott, W. Germany). The signal from the photomultiplier was directly displayed either on a servo recorder (Hitachi Model 056, Japan) or stored in a digital storage oscilloscope (Iwatsu SRI 100, Japan). The signal was triggered with the help of an electric shutter with an opening time of 10 ms. The fronds were placed in a acrylic holder and placed diagonally in a 4 ml glass cuvette to face the photomultiplier at  $45 \text{ }^\circ\text{C}$ . The fronds were incubated in dark for 10 min prior to blue light excitation as described by [12].

### Statistical analysis

Treatment means were compared by analysis of variance using the statistical package SPSS. Each sampling time was analysed separately. The data were processed by single factor analysis of variance. LSD was calculated at 5% level of probability.

## RESULTS

### 4.1 Effect of short-term UV-B treatment on growth characteristics

*Lemna* fronds grown in troughs containing the growth factors were subjected to photoperiod of 10-12 h and subjected to UV-B treatment (30 min/day) at an irradiance of  $5 \text{ W m}^{-2}$  for various time periods (2 to 12 days with an interval of 2 days). The biomass, doubling time and relative growth rate of *Lemna* after 12 days of UV-B treatment is presented in Table 1. After a prolonged 12 days of UV-B treatment, the biomass was reduced by 37%. Similarly, the doubling time of *Lemna* increased from 8.5 to 8.8 days with a decrease in RGR (relative growth rate). Supplementation of UV-B caused significant decrease in the vegetative growth of *Lemna* fronds.

### 4.2 Effect of short-term UV-B treatment on pigment composition

Changes in photosynthetic and non-photosynthetic pigment composition of *Lemna* fronds exposed to short-term UV-B for various time periods are shown in Fig.1(a,b,c,) and Fig. 2(a,b). On a unit fresh weight basis, UV-B irradiation had caused a 7-13% decrease in Chl *a* during the initial period (2-4 days) and later 5-15% increase after 6-8 days of UV-B exposure was noticed (Fig.1a). Prolonged exposure (10-12 days) resulted in 18% decrease in Chl *a* content. Compared to Chl *a*, the amount of Chl *b* remained more or less constant up to 6 days of UV-B treatment (Fig.1b). Though marked changes were not observed in Chl *a+b* ratio in the early stages, a slight increase was observed after 6 days (Fig.1c). A similar trend was observed in carotenoid levels also. Significant decrease in carotenoid content was observed only after 6 days of UV-B exposure (Fig.1d). In contrast to photosynthetic pigments, flavonoid content showed a tendency to increase under UV-B radiation Fig. 1e.

### 4.3 Effect of short-term UV-B on photosynthetic activities

PSII mediated photosynthetic  $\text{O}_2$  evolution measured in control and UV-B irradiated in intact *Lemna* fronds revealed that there was a significant increase in  $\text{O}_2$  evolution in control throughout the growth period. A maximum decrease in photosynthetic activity was observed after 12 days of growth under UV-B radiation. Table 2 provides the PSII mediated  $\text{O}_2$  evolution rates in which a maximum decrease of 68% in PSII activity was noticed after 12 days of growth under UV-B.

**Table 1 Effect of short-term UV-B radiation on biomass, doubling time, and relative growth rate in *Lemna***  
(Values are Mean  $\pm$  SE of 5 samples). UV-B irradiation time was 30 min/day. Values shown in parentheses are percent over control.

Parameters	Control	+UV-B
Biomass (g)	1.20 $\pm$ 0.30	0.75 $\pm$ 0.18 (63)
Doubling time (d)	8.5 $\pm$ 1.00	8.8 $\pm$ 1.20 (103)
Relative growth rate ( $\text{kg kg}^{-1}\text{d}^{-1}$ )	0.05 $\pm$ 0.005	0.04 $\pm$ 0.005 (80)

**Table 2 Changes in PSII mediated  $\text{O}_2$  evolution (Photosynthetic activity) *Lemna gibba* fronds exposed to short-term UV-B radiation (30min / d) for different time periods**

The values are an average of 5 independent measurements Mean  $\pm$  SE, n=5. The level of UV-B irradiation at the frond surface was  $5 \text{ W m}^{-2}$ . Values in parentheses are percent over controls.

Treatment period (days)	PSII activity ( $\mu\text{mol O}_2/\text{g}^{-1}\text{fr}/\text{m}/\text{h}^{-1}$ )	
	Control	+ UV-B
2	28.8 $\pm$ 1.2	27.2 $\pm$ 0.9 (94.4%)
4	30.2 $\pm$ 1.4	25.2 $\pm$ 1.1 (83.4%)
6	32.5 $\pm$ 1.6	18.36 $\pm$ 0.9 (56.5%)
8	33.5 $\pm$ 1.8	14.4 $\pm$ 1.0 (43.2%)
10	30.2 $\pm$ 1.0	10.8 $\pm$ 0.7 (36%)
12	22.5 $\pm$ 0.9	7.2 $\pm$ 0.6 (32%)

To study the influence of short term UV-B radiation on the primary photochemical events, Chl *a* fluorescence induction was measured in intact fronds. Typical fast and slow Chl *a* fluorescence transients recorded with intact *Lemna* fronds are shown in Fig. 3. The initial fluorescence ( $F_0$ ) level remained unaffected under all treatments whereas a significant change in the variable fluorescence ( $F_v$ ) was noticed under UV treatment. During the first 4 days of UV-B treatment, the  $F_m$  level was found to be unaltered and thereafter decreased gradually. With increase in

the time of UV-B treatment, the D-P rise was found to be missing with reduction in  $F_v$  level. Unaffected  $F_0$  level indicates that the PSII antenna chlorophylls are not disturbed by UV-B treatment whereas increase or decrease in  $F_m$  indicates the functioning efficiency of PSII reaction centers resulting in complete  $Q_A$  reduction. The latter conclusion is also supported by the fast D-P rise in UV-B treated fronds. Since  $F_v/F_m$  is a measure of photochemical efficiency, quantification of  $F_v/F_m$  ratios would reveal the photochemical efficiency of PSII directly (18). The  $F_v/F_m$  values are shown in Table 3 which increases in UV-B treatment period, the  $F_v/F_m$  values were found to decrease with maximum reduction after 10 days of exposure. In contrast to fast kinetics, the slow transients (Fig. 3) included P, M, S and T. In control fronds, P-S quenching was fast even after 3 days of exposure. The 'M' peak was prominent in control and treated fronds and reduced slowly with increase in age. In the case of UV treatment, absorbance of S-M rise and early attainment of T state was noticed.

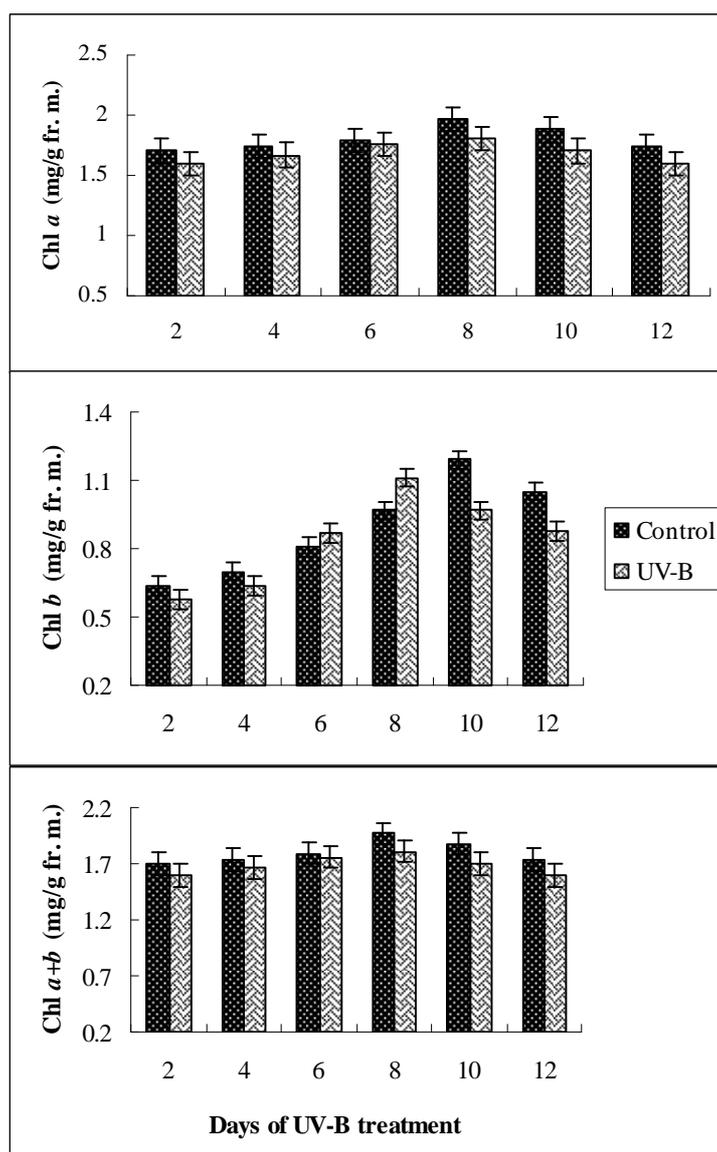


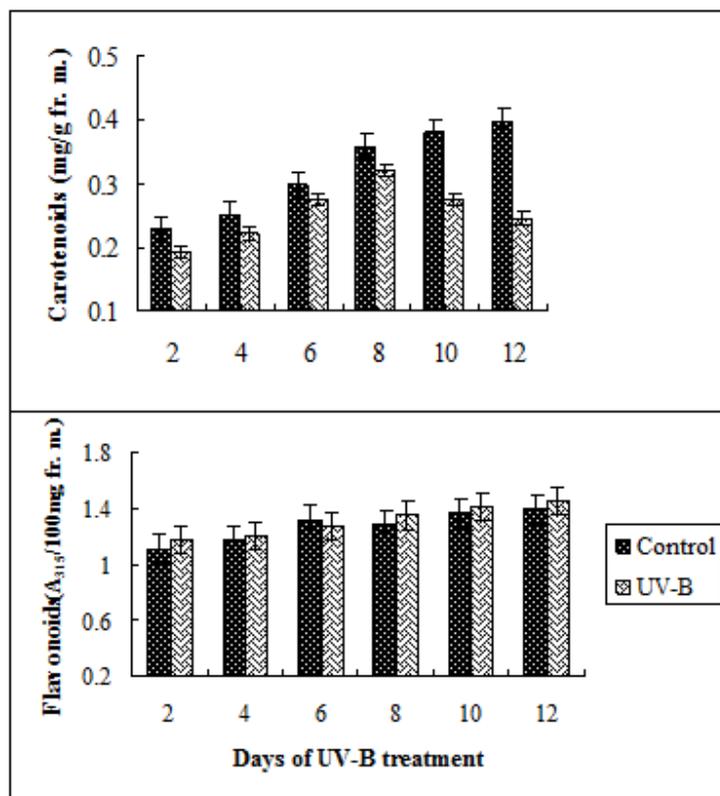
Fig. 1. Changes in Chl *a*, *b* and total Chl level of *Lemna* fronds exposed to short-term UV-B treatment (30min/day) at 5W m<sup>-2</sup> for various time periods

The values are expressed on unit fresh weight basis. Mean  $\pm$  SE,  $n=5$ .

**Table 3 Quantitative changes in Fv/Fm values obtained from fast fluorescence transients of *Lemna* fronds exposed to short-term UV-B radiation**

The values are an average of 5 independent tracings. Means  $\pm$  SE, n=5.

Treatment period (days)	Fv/Fm ratio	
	Control	+UV-B
2	0.72 $\pm$ 0.02	0.70 $\pm$ 0.01
4	0.77 $\pm$ 0.02	0.74 $\pm$ 0.02
6	0.81 $\pm$ 0.02	0.77 $\pm$ 0.04
8	0.84 $\pm$ 0.03	0.79 $\pm$ 0.04
10	0.72 $\pm$ 0.05	0.66 $\pm$ 0.02
12	0.67 $\pm$ 0.04	0.57 $\pm$ 0.04



**Fig. 2. Changes in non-photosynthetic pigment composition (carotenoids and flavonoids) of *Lemna* fronds exposed to short-term UV-B treatment (30min/day) at an irradiance of 5W m<sup>-2</sup> for various time periods**

Mean  $\pm$  SE, n=5.

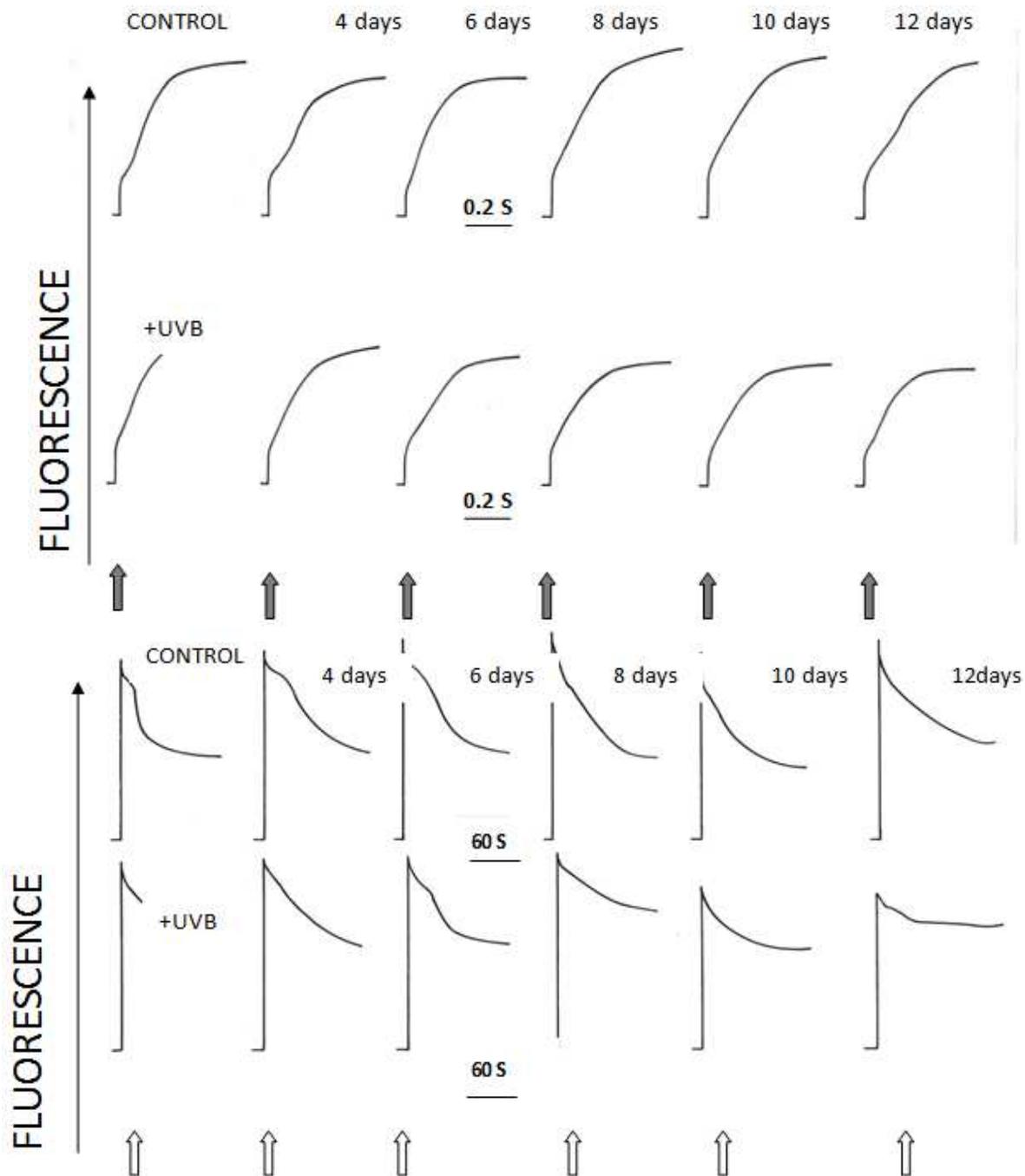


Fig.3. Typical fast and slow fluorescence transients obtained with control and short-term UV-B ( $5 \text{ W m}^{-2}$  for 30 min/day) irradiated *Lemna* fronds

Tracings were obtained with three individual samples.

### DISCUSSION

These results correspond to earlier field studies with terrestrial system [5]. Morphological and physiological responses of plants to UV-B radiation such as decreased leaf area serve to limit light interception by photosynthetic tissue and therefore protect against photo inhibition. It is well known that UV-B radiation inhibits vegetative growth which is attributed to the destruction of endogenous IAA [13] [14] and induction of oxidative enzymes associated

with growth responses. Enhanced UV-B was shown to cause damage to Chl *b* rather than Chl *a* which could be due to its direct absorption or due to inhibition in the Chl biosynthesis [15].

Flavonoid accumulation is dependent on the nature of the irradiation programme (which involves simultaneous and sequential irradiation) in which UV-B is obligatory in inducing the response [16]. The induction pattern observed here supported the above hypothesis. Synthesis of phenolic secondary metabolites is known to protect plants from UV-B radiation [17].

Thus, accumulation of UV-B absorbing compounds in *Lemna* epidermis was suggested to be a protective mechanism against UV-B radiation. The fronds exhibited a typical OI-DPSMT fluorescence induction pattern [18]. The rate of PSII activity measured in terms of O<sub>2</sub> evolution did not correlate with the rate of PSII as measured by fast fluorescence could be due to the fact that F<sub>v</sub>/F<sub>m</sub> values recover rapidly in the dark [19]. The slow transients of Chl *a* fluorescence exhibit an interplay between overall electron transport and carbon reduction cycle [20] the transport of electron from Q to PS I make the chlorophyll fluorescence to decline from maximum level P to S. Slow P-S quenching in treated fronds could be due to low rate of PSI activity and subsequently, early attainment of T state due to reduction in overall photosynthesis. The most activated enzyme under light is the terminal enzyme of the electron transport chain, the Fd<sup>2+</sup>-NADP reductase which leads to the decline of fluorescence level from P to S [21].

Early attainment of T state in UV treated fronds reflects the low efficiency of PSII and CO<sub>2</sub> fixation pathway [22]. *Azolla* fronds exposed to UV-B radiation showed a radiation in variable (I-P) fluorescence yield. This indicates the damage of PS2 activity, particularly at the donor site [10]. Foliar symptoms of UV-B treatment like bronzing, scorching, glazing or chlorosis have been shown to occur in many plants [23] [24] 1981). Growth characteristics such as shoot height, root length and leaf area were found to be altered in higher plants exposed to UV-B. Decreased in stem length, leaf area and plant height by artificial or natural UV-B radiation was observed in cucumber, sun flower and soybean (Sullivan and Teramura, 1989). The sensitivity of an important aquatic macrophyte, duckweed (*Lemna major*), to UV-B radiation was observing visible injury symptoms and estimating levels of chlorophyll, pheophytin, carotenoids, protein, starch, free sugar, and peroxidase activity. Peroxidase activity increased at all the exposure levels. Dose-dependent decrease in chlorophyll and starch with drastic depletion in protein and free sugar content were observed. Pheophytin and carotenoids content increased at no injury level, but decreased at higher exposure level. Farooq (2000) reported the results indicate that ambient UV-B radiation at the indicated level acts as a physiological stress in *Lemna major*.

Thus short- term UV-B exposure in the present study reveals the severity of *Lemna* fronds with reduction in photosynthetic pigment composition and photosynthetic activity proving that aquatic species are more sensitive than terrestrial plants.

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