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Effects of ultraviolet radiation on pigmentation and malondialdehyde content of three aquatic macrophytes

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ABSTRACT

A study has been done in order to evaluate the ill effects of UV-A,UV-B and UV-C on pigmentation and malondialdehyde content of floating macrophytes (*Lemna* sp., *Pistia* sp. and *Eichhornia* sp.) in one, three, and five days interval. Study results revealed that all types of ultraviolet light (UV-A, UV-B, and UV-C) did not produce same extent of ill effects on the studied macrophytes. *Pistia* sp. and *Eichhornia* sp. showed similar reduction pattern of chl a/chl b ratio with respect to control. Results also suggest that among the three types of radiation only UV-B showed higher level of changes in both the pigment and malondialdehyde content. Moreover, among the three tested macrophytes only *Lemna* sp. showed some protective role against UV radiation compared to other to macrophytes.

Keywords: Floating macrophytes, *Lemna* sp., *Pistia* sp., *Eichornia* sp., UV radiation, Pigment content, malondialdehyde

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INTRODUCTION

Ultra violet radiation (UV) is a part of non-ionizing radiation of electromagnetic spectrum that comprises about 8-9% of the total solar radiation (Coohill 1989), (Frederick 1993). There are three distinct category of UV radiation. Every category has specific wavelength ranges, UV-A (320-400 nm); UV-B (280-320nm), and UV-C (200-280 nm). So far as ill effect is concern, the UV-C radiation is considered as extremely harmful to living organisms. UV-B is of special interest because of its damaging effects in plants. However, UV-B represents only approximately 1.5% of the total spectrum. On the other hand, UV-A represents approximately 6.3% of the incoming solar radiation and it is considered least hazardous part of UV radiation (Hollosy 2002). Thinking about the bad effects of UV radiation was intensified after the news of the destruction of ozone layer. As we know that ozone layer acts as a key component protecting living beings from the damaging of UV radiation. There are numerous anthropogenic factors such as release of chlorofluorocarbon in to the environment, results destruction of ozone layer ~5% (Pyle 1996). However, Hollosy (2002) reported that, in general, about 1% reduction of ozone layer results in a 1.3-1.8% increase in the amount of biologically active UV-B radiation.

Numerous researchers (Sarghein et al. 2011, Nawkar et al. 2013) focused on the effect of plant growth and stress physiology under artificial UV-B radiation in growth chamber. The current knowledge regarding eco-physiological impact of UV radiation on plants has come largely through field experiments using natural or moderately higher levels of UV-B radiation (Nawkar et al. 2013). It has been well documented that plants respond differentially to UV influence rate as well as wave length (Ulm et al. 2004, Frohnmeyer and Staiger 2003). Lower doses of UV-B stimulate photomorphogenesis in etiolated plants while higher doses of UV-B or UV-C result in cellular damage (Frohnmeyer and Staiger 2003, Suesslin and Frohnmeyer 2003). It was also reported that over exposure of UV-C can induce programmed cell death through activation of proteases, oligonucleosomal DNA fragmentation, and appearance of apoptotic nuclear morphology in *Arasidopsis thaliana* (Danon and Gallois 1998, Gao et al. 2008). Moreover, UV-B exposure can also induce programmed cell death in a BY-2 tobacco cell line (Lytvyn et al. 2010). Therefore, from the previous literature it is clear that very limited work has been done only on the ill effects of UV radiation on pigmentation.

Keeping in view on the above fact, present work is dedicated on the radiation effects of UV-A, UV-B, and UV-C on the both pigment and malondialdehyde of three floating macrophytes.

MATERIAL AND METHODS

Collection of macrophytes. Three types of macrophytes, *Lemna* sp., *Pistia* sp., and *Eichhornia* sp. were collected from Golapbag campus, Burdwan University, Burdwan, West Bengal. After collection of macrophytes, it was thoroughly washed with double distilled water and removes the dart. The washed macrophyte was weighted after shocking the water with tissue paper. The fixed weight (10 g) of each macrophyte was taken in a set of plastic bowl with 250 ml lake water collected from the save lake from where the studied macrophyte was collected.

Exposure of UV radiation. All the studied macrophyte was exposed with UV radiation of different wave length such as 254 nm, 312 nm, and 365 nm for 1 h in each day during whole experimental period. The macrophytes were exposed at first, third and fifth days of incubation.

Estimation of chlorophyll and carotenoid contents. Fresh young leaves (0.1 g) were selected from macrophytes under each treatment at the last day of the experiment, and washed with deionized water. The leaves were cut into small pieces. Chlorophyll fractions 'a', 'b' and total chlorophyll were determined in the acetone extract (80% v/v) (Bates 1973) measured in a spectrophotometer at 645, 652 and 663 nm, respectively and carotenoid content estimated by MaClachlan and Zalik (1963). The concentrations were expressed as mg chlorophyll g^{-1} fresh weight with the following equations:

Chl "a" (mg g^{-1} fw)=[$12.7*D_{663}$–$2.69*D_{645}$]*VW/1000

Chl "b" (mg g^{-1} fw)=[$22.9*D_{645}$–$4.68*D_{663}$]*VW/1000

Total Chl (mg g^{-1}fw)=D_{652}*1000*VW/1000

Total Carotenoid content (mg g^{-1})=[$7.6*D_{480}$–$1.49*D_{510}$]*VW/1000

where D = optical density; V = final volume of 80% acetone; W = weight of sample; f.w. = fresh weight of the sample.

Determination of MDA content. Homogenize 0.1 mg of leaf tissue by adding 10 ml 0.1% (w/v) TCA then Centrifuge the homogenate for 10 min (15,000 x g, 4 °C) then Collect supernatant and mix 1 ml of supernatant with 4 ml 0.5% TBA diluted in 20 % TCA. Incubate in water bath at 95 °C for 30 min. End reaction by incubating on ice. In case the solution is not clear, centrifuge for a further 10 min (10,000 x g, 4 °C) and measure the absorbance at 532 and 600 nm (Heath and Packer 1968). MDA content was calculated

by the equation: MDA equivalents ($nmol.cm^{-1}$) = 1000[(Abs 523 – Abs 600nm)/155].

Statistical analysis. The entire data were statically interpreted by following the Pearson correlation study with different p value for significant test. The statistical analysis was done by using statistical software Minitab 16.

RESULTS AND DISCUSSION

Present study results demonstrated the total chlorophyll level of the entire studied macrophytes showed little unusual picture (Table 1). Initially total chlorophyll level was low after first day of UV exposure, but after third day of UV exposure total chlorophyll level was little improved followed by drastic reduction was recorded after fifth day of UV exposure. In addition, the gradual reduction of pigments in the form of Chlorophyll 'a' (chl 'a'), Chlorophyll 'b' (chl 'b'), total chlorophyll, carotenoid under the influence of UV-A, UV-B, and UV-C. Especially UV-B exposure reduced the chl 'a' and chl 'b' in different extent in different macrophytes. Studied macrophytes *Lemna* sp. and *Pistia* sp. showed higher reduction in chl a compared to chl 'b'. However, *Eichhornia* sp. showed higher reduction in chl 'b' (33.53%) compared to chl 'a' (31.28%). Similar degradation pattern of pigment was highlighted by Marwood and Greenberg (1996) and they reported that UV-B radiation resulted in greater reduction in the amount of chl 'b' as opposed to chl 'a' and may point to a more selective destruction of chl 'b' biosynthesis or degradation of precursors. Almost similar observation reported by Strid et al. (1990) and Jordan (1996). They focused in their study that pigment of the photosynthetic apparatus can be destroyed by UV- radiation, with concomitant loss of photosynthetic capacity. Again Pfundel et al. (1992) reported that chlorophylls (chl) and carotenoid may be adversely affected by relatively large amount of UV-B radiation, with carotenoid generally being less affected than the chlorophylls.

It is clear that all the three macrophytes (*Lemna* sp., *Pistia* sp., and *Eichhornia* sp.) showed different chl 'a'/chl 'b' ratio (Table 2). During first day exposure of UV-A, the average chl 'a'/chl 'b' ratio reduced maximum for *Lemna* sp. followed by *Eichhornia* sp. and less reduction for *Pistia* sp. with respect to the control. Interestingly other two macrophytes namely *Pistia* and *Eichhornia* sp. Showed similar chl 'a'/chl 'b' reduction pattern with respect to control. During three days of incubation chl 'a'/chl 'b' ratio increase for *Lemna* sp and *Eichhornia* sp. under influence of UV-A and UV- C. However, UV-B showed unchanged chl a/chl b ratio for *Pistia* sp. During fifth day of exposure *Lemna* sp. and *Pistia* sp. showed much higher leve reduction of chl 'a'/chl 'b' ratio than *Eichhornia* sp. under all three types (UV-A, UV-B, and UV-C) of radiation. However, maximum reduction was observed under UV-B radiation for all three types of macrophytes. Almost similar observation was reported by Smith et al. (1992) they reported that marine phyto plankton showed about 6% to 12% reduction of photosynthetic productivity due to increased solar UV-B radiation under the Antarctic ozone hole. However, Donkor and Hader (1995) reported in their paper that UV-B radiation can cause structural changes in the photosynthetic apparatus leading to inhibition of photosynthesis. Such structural changes in the photosynthetic apparatus have also been detected in higher plants after exposure to solar radiation (Renger et al. 1989, Tevini et al. 1989). Thus, UV-B radiation has been found to damage the reaction center of photosystem-II and to cause structural changes in the D1 and D2 proteins in the thylakoid membranes (Donkor and Hader 1996).

On the other hand carotenoid showed similar reduction trends with UV exposure in all macrophytes except *Lemna* sp. during first, third and fifth days incubation (Table 3). Therefore, it can be concluded that *Lemna* sp. has some protective role against ultraviolet radiation compared to *Pistia* sp. and *Eichhornia* sp. On the other hand Donkor and Hader (1996) reported that carotenoid can play dual functions as photoprotection and energy transduction. The same is also reported by Hader and Hader (1990) and Zündorf and Harder (1991).

The malondialdehyde (MDA) levels of all studied macrophytes increased after exposure of ultraviolet radiation, but extent of MDA enhancement is different for different macrophytes. Under exposure of UV-A and UV-C MDA content of *Lemna* sp. Significantly increased with respect to control in third and fifth days of incubation. But MDA content reduced in third day of incubation under UV-B exposure (Table 4). On the other hand *Pistia* sp. showed gradual incremental pattern of MDA under three types of UV radiation in first, third and fifth days of exposure. However, *Eichhornia* sp. showed fluctuating nature of MDA has under UV-A, UV-B and UV-C exposure. Such increment of MDA level also reported by Peykarestan et al. (2012). Under UV irradiation of some plants species such as Portulaca grandiflora and Portulaca oleracea. Again Rogozhin et al. (2000) highlighted that prolonged irradiation of seeds with UV light (1-6 h) led to an increase in the level of lipid peroxidation in wheat sprouts. This suggested a breakdown of acylglycerols during radiation processing, resulting in the release of free fatty acids.

Table 1. Total chlorophyll content of three aquatic macrophytes under UV radiation in different day intervals.

Trat.	First Day			Third Day			Fifth Day		
	Lemna sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.
control	0.83 ± 0.01	1.82 ± 0.01	1.95 ± 0.06	0.25 ± 0.01	1.20 ± 0.01	1.88 ± 0.03	0.66 ± 0.10	0.92 ± 0.01	1.72 ± 0.01
UV-A	1.94 ± 0.02	0.99 ± 0.01	0.98 ± 0.08	1.70 ± 0.01	2.16 ± 0.01	1.52 ± 0.05	0.55 ± 0.11	2.87 ± 0.01	1.03 ± 0.10
UV-B	0.38 ± 0.05	1.37 ± 0.03	0.85 ± 0.01	2.01 ± 0.03	1.88 ± 0.02	1.06 ± 0.07	0.41 ± 0.11	5.15 ± 0.01	1.30 ± 0.01
UV-C	0.41 ± 0.01	1.17 ± 0.01	0.66 ± 0.01	2.56 ± 0.05	1.76 ± 0.01	1.56 ± 0.01	0.68 ± 0.10	1.51 ± 0.12	0.85 ± 0.01

Mean ± standard deviation.

Table 2. Chlorophyll a / chlorophyll b ratio of three aquatic macrophytes under UV radiation in different day intervals.

Trat.	First Day			Third Day			Fifth Day		
	Lemna sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.
control	1.96 ± 0.11	2.11 ± 0.10	2.30 ± 0.09	1.95 ± 0.05	2.06 ± 0.03	2.28 ± 0.03	1.91 ± 0.02	2.08 ± 0.02	2.28 ± 0.01
UV-A	1.14 ± 0.02	1.77 ± 0.20	1.34 ± 0.06	1.77 ± 0.11	1.76 ± 0.06	1.70 ± 0.08	0.90 ± 0.04	1.50 ± 0.01	1.90 ± 0.08
UV-B	1.22 ± 0.02	1.86 ± 0.12	1.51 ± 0.01	1.33 ± 0.10	1.75 ± 0.02	1.35 ± 0.07	1.33 ± 0.08	0.91 ± 0.01	1.59 ± 0.05
UV-C	1.19 ± 0.02	1.87 ± 0.11	1.45 ± 0.22	1.39 ± 0.05	1.71 ± 0.01	1.50 ± 0.08	0.98 ± 0.06	1.28 ± 0.05	1.71 ± 0.01

Mean ± standard deviation.

Table 3. Carotenoid content of three aquatic macrophytes under UV radiation in different day intervals.

Trat.	First Day			Third Day			Fifth Day		
	Lemna sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.
control	0.27 ± 0.04	0.43 ± 0.01	0.59 ± 0.03	0.25 ± 0.10	0.41 ± 0.02	0.53 ± 0.01	0.21 ± 0.01	0.24 ± 0.01	0.44 ± 0.09
UV-A	0.13 ± 0.01	0.25 ± 0.10	0.34 ± 0.02	0.11 ± 0.01	0.24 ± 0.07	0.32 ± 0.01	0.13 ± 0.01	0.19 ± 0.01	0.29 ± 0.01
UV-B	0.35 ± 0.02	0.37 ± 0.11	0.31 ± 0.01	0.30 ± 0.01	0.33 ± 0.10	0.29 ± 0.01	0.26 ± 0.01	0.26 ± 0.07	0.28 ± 0.05
UV-C	0.24 ± 0.01	0.31 ± 0.01	0.27 ± 0.01	0.16 ± 0.02	0.23 ± 0.01	0.19 ± 0.03	0.08 ± 0.01	0.22 ± 0.08	0.16 ± 0.03

Mean ± standard deviation.

Table 4. Malondialdehyde content of three aquatic macrophytes under UV radiation in different day intervals.

Trat.	First Day			Third Day			Fifth Day		
	Lemna sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.	*Lemna* sp.	*Pistia* sp.	*Eichhornia* sp.
control	0.20 ± 0.01	0.23 ± 0.01	0.36 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.29 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.20 ± 0.01
UV-A	0.16 ± 0.01	0.21 ± 0.05	0.61 ± 0.01	0.49 ± 0.02	0.42 ± 0.06	0.26 ± 0.33	0.22 ± 0.02	1.03 ± 0.03	0.50 ± 0.01
UV-B	0.20 ± 0.01	0.22 ± 0.01	0.68 ± 0.01	0.16 ± 0.07	0.61 ± 0.01	0.14 ± 0.02	0.28 ± 0.02	1.06 ± 0.20	1.06 ± 0.01
UV-C	0.19 ± 0.01	0.14 ± 0.03	0.80 ± 0.03	0.45 ± 0.06	0.23 ± 0.01	0.06 ± 0.01	0.28 ± 0.01	1.67 ± 0.01	0.43 ± 0.01

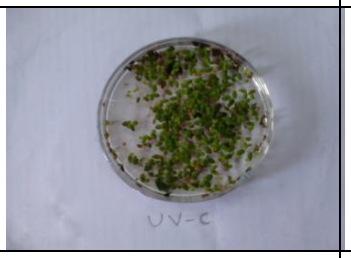
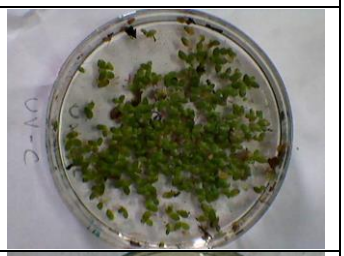
Mean ± standard deviation.

Table 5. Correlation between different characteristics of three aquatic macrophytes under UV radiation in different day intervals.

Days of incubation	Species	Parameters	Correlation	Significant level
1st day	*Lemna* sp.	Chl b vs Chl a	1.000	P < 0.009
		MDA vs Chl a	-1.000	P < 0.007
		MDA vs Chl b	-1.000	P < 0.002
3rd day		MDA vs Chl b	0.999	P < 0.023
		MDA vs Carotenoid	-0.990	P < 0.091
5th day		Carotenoid vs Chl a	-0.998	P < 0.037
1st day	*Pistia* sp.	Chl b vs Chl a	0.998	P < 0.038
		Carotenoid vs total Chl	1.000	P < 0.002
3rd day		Chl b vs Chl a	0.982	P < 0.119
5th day		Chl b vs Chl a	-0.999	P < 0.026
1st day	*Eichhornia* sp.	Total chl vs Chl a	0.992	P < 0.082
		Carotenoid vs Chl a	0.992	P < 0.080
		Carotenoid vs total Chl	1.000	P < 0.002
		MDA vs total Chl	-1.000	P < 0.020
3rd day		Carotenoid vs Chl a	1.000	P < 0.018
5th day		Total chl vs Chl b	0.953	P < 0.196
		Carotenoid vs Chl a	0.990	P < 0.089

Figure 1. Photographic images of three aquatic macrophytes under UV radiation in different day intervals.

UV rad	Species	1st day	3rd day	5th day
Control	*Lemna* sp.			
	Pistia sp.			
	Eichhornia sp.			
UV-A	*Lemna* sp.			
	Pistia sp.			
	Eichhornia sp.			
UV-B	*Lemna* sp.			

	Pistia sp.			
	Eichhornia sp.			
UV-C	*Lemna* sp.			
	Pistia sp.			
	Eichhornia sp.			

Experimental demonstration clearly revealed that greenness of the studied macrophytes reduced after exposure of UV-radiation (Figure 1). It is clear that all the three tested macrophytes detoriated after fifth days radiation of both UV-A and UV-B. However, UV-C radiation dose not showed such high level chlorophyll reduction except *Pistia* sp. Moreover three tested macrophytes showed the degradability order as *Lemna* sp. > *Eichhornia* sp. > *Pistia* sp. Under UV-B radiation.

Overall correlation study (Table 5) indicated *Lemna* sp. has strong significant negative relationship between MDA content and chl 'a'($p < 0.007$) and chl 'b' ($p < 0.002$); MDA content and carotenoid ($p < 0.091$) and chl 'a' and carotenoid ($p < 0.037$) during first, third, and fifth days of UV exposure respectively. However, in first and third days exposure, *Lemna* sp. showed significant positive relationship between chl'b' and chl'a' ($p < 0.009$) and MDA content and chl'b' ($p < 0.023$) respectively. But *Pistia* sp. and *Eichhornia* sp. showed strong negative relationship between chl'a' and chl'b' ($p < 0.026$); MDA content and total Chlorophyll ($p < 0.020$) during fifth and first days of UV exposure respectively.

CONCLUSIONS

Present research highlighted that only UV-B radiation is detrimental with respect to the reduction of both

green pigment and MDA. Among the three tested macrophytes only *Lemna* sp. showed some protective role against UV radiation compared to other macrophytes. Finally, it can be concluded that although UV-B radiation has negative impact on system but it also caused substantial hazards due to its (UV) penetration into the deeper euphotic zone than considered before. Finally, long-term studies are necessary to understand the effects on long-lived perennials that might accumulate damage through time, and on populations or communities of plants.

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