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Interactive Effects of UV-B and Pesticides on Photosynthesis and Nitrogen Fixation of *Anabaena doliolum*

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Abstract The effects of UV-B and commercial grade pesticides (butachlor and carbofuran), individually and in combination, were studied on a variety of physiological processes of rice field cyanobacterium *Anabaena doliolum*. Butachlor was found to be 2–12% more toxic than carbofuran and 4–24% than UV-B on the growth, photosynthesis, lipid peroxidation, membrane permeability, and nitrogenase activity of the test cyanobacterium. Of the three photosynthesis inhibitors, the butachlor-induced inhibition of whole chain was approximately 3 and 21% higher than carbofuran and UV-B, respectively. Although the interaction of the stress factors caused a significant inhibition ($P<0.01$), it was still less than the additive effect on the parameters investigated, except for PSI.

Key words: *Anabaena doliolum*, UV-B, pesticides, photosynthesis, nitrogen fixation

Of the various kinds of environmental stresses confronting human beings, UV-B and pesticides assume a special significance. The continued depletion of the stratospheric ozone, due mainly to anthropogenically generated atmospheric pollutants such as chlorofluorocarbons (CFCs), is responsible for an increase in solar UV-B (280–315 nm) radiation reaching the Earth's surface [13]. In addition to the Antarctic ozone hole, where UV radiation has been detected down to a depth of 70 m, ozone depletion has also been reported in the north polar region [13].

UV-B radiation has been reported to reduce growth rate, carbon fixation, O_2 evolution, photosynthetic electron transport, and the ATP pool size of cyanobacteria [4, 12, 15, 24, 27]. UV-B has sufficient energy to induce photodamage in proteins, nucleic acids, lipids, and other biological compounds [38]. UV-B has been reported to induce a significant reduction in the maximum quantum yield of the PS II (V_m/F_m) and minimal fluorescence (F_o) of the diatom, *Gomphonema baltium* [11]. UV-B is also known to produce oxidative stress [2].

In order to meet the food requirements of a rapidly increasing population, current agricultural techniques involve regular treatment of crops, including rice, with suitable herbicides and insecticides for protection against pests. These pesticides are nontarget in their action and thus kill a host of cyanobacteria, many of which fix atmospheric nitrogen and help increase the soil fertility. Pesticides are known to affect microflora, by inhibiting enzyme activity, altering cell membrane permeability and photosynthesis, and interfering with the synthesis of DNA, RNA, and proteins [17]. Herbicides cause a decrease in the chl *a* content, ratio of chl (*a*/*b*), and growth rate and dry weight of *Scenedesmus* sp. [9]. Herbicides also affect photosynthesis by reducing the electron transport of *Halophila ovalis* [25, 28]. Different species of cyanobacteria show a varied tolerance to commercial grade furadan (3% carbofuran) [29]. The survival, growth, and nitrogen fixation of *Anabaena* sp. and *Westiellopsis prolifica* are inhibited by the insecticide sevin (50% w/v) above 50 μg/ml [1]. The herbicide butachlor causes a decline in the protein and phycobiliprotein levels, and inhibits heterocyst differentiation and nitrogen fixation [17]. In addition, the herbicides butachlor and fluchloralin affect the oxygen evolution, nitrogenase, nitrate reductase, and glutamine synthetase activities of *Nostoc muscorum* [33].

Soil, as the ultimate sink in the rice field, harbors many beneficial microorganisms, including cyanobacteria, which are potentially susceptible to pesticides. Since a considerable amount of UV-B radiation is now reaching the surface of Earth, including rice fields, there is every likelihood of an interaction between UV-B and the pesticides used in rice fields, which is a pertinent although unexplored environmental issue. Accordingly, the current study is the first attempt to address this question. As regards the damage to cyanobacteria by UV-B and pesticides, it is hypothesized

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that UV-B and pesticides may (i) produce severe effects on the photosynthesis and nitrogen fixation of cyanobacteria, (ii) interfere with the membrane behavior, and (iii) inhibit the metabolic processes of test cyanobacteria in a synergistic, antagonistic, additive, or different manner. Therefore, to test the above hypotheses, the impact of UV-B and pesticides was studied individually as well as in combination on the growth, photosynthesis, carbon fixation, ATP content, nitrogen fixation, membrane permeability, and lipid peroxidation of *Anabaena doliolum*.

MATERIALS AND METHODS

Organisms and Growth Conditions

Anabaena doliolum Bharadwaja was isolated from the rice field at Banaras Hindu University campus and maintained in the laboratory in a modified Chu-10 medium [10] at 24±2°C under a 72 μmol photon m^{-2} s^{-1} PAR (photosynthetically active radiation) light intensity and photo-period of 14:12 h with regular shaking. Exponentially grown cells were used in all the experiments.

Pesticide Stock Solution

Butachlor (herbicide) and carbofuran (insecticide) were selected for the current study as they are the most commonly used pesticides in rice paddy fields. Butachlor, locally known as machete (butachlor, EC 50%), is a pre-emergent herbicide used to control the weeds in seeded and transplanted rice. One ml of commercial grade butachlor (EC 50%) was mixed with 99 ml of double distilled water to make a 100 ml stock solution. Carbofuran, locally known as furadan (carbofuran, 3% G), belongs to the carbamate group of pesticides. It is a systemic insecticide, acariade, used to control soil and foliar feeding insects. One gram of commercial grade carbofuran (3% granular) was stirred with 10 ml of acetone for thorough mixing and then increased to 100 ml with double distilled water to make a stock solution. To avoid bacterial contamination, the stock solutions were Millipore filtered just before use.

UV-B Radiation

The UV-B radiation was provided by a UV-B lamp (No. 3-4408, Fotodyne Inc) at a maximum output of 310 nm. The UV-B dose (12.9 mWm^{-2} nm^{-1}) selected was based on the latitude of authors work station (25°N) and the mean percentage depletion of the ozone layer calculated by Smith *et al.* [36]. The above radiation dose was obtained by adjusting the distance between the UV-B source and the cyanobacterial suspension (10 ml) kept in an open glass petri dish. The algal suspension was stirred continuously with the help of a magnetic stirrer to allow uniform exposure.

Measurement of Survival and Growth

Exponentially growing cyanobacterial cells exposed to UV-B for 0–1 h were withdrawn at regular intervals, and 0.05-ml aliquots of an algal suspension containing 5.3×10^4 cells ml^{-1} were plated onto agar plates. Likewise, to measure their survival against pesticides, the cells were plated onto agar plates containing different concentrations of carbofuran (50, 100, 200, 300, 400, 500, 1,000 ppm) and butachlor (5, 10, 50, 100, 150, 200 ppm). The selection of the above range of pesticides was based on information available in the literature [30, 33] as well as the concentrations used in the field. The LC_{50} doses for both the UV-B and the pesticides were determined by the plate colony count method [26] after 2 weeks of treatment. A 25 (LC_{25}) and 50% (LC_{50}) survival of the cyanobacterium was recorded after 12 and 26 min of UV-B exposure, respectively. In the case of the pesticides, the LC_{25} and LC_{50} doses for butachlor were 4 and 10 ppm, and 120 and 300 ppm for carbofuran, respectively. Growth was expressed in terms of protein content [21] up to the 15th day.

Measurement of O_2 Evolution and Photosynthetic Electron Transport Chain

The oxygen evolution was measured at 25°C at an illumination of a 300 μmol m^{-2} s^{-1} PAR light intensity using a clark type O_2 electrode (digital oxygen system, Model-10, Rank Brother, U.K.). For the O_2 evolution, a 5-ml algal culture was taken into the vessel and the O_2 evolved was measured up to 5 min under the light.

The photosynthetic electron transport activity was determined by three basic assays. A cell-free thylakoid membrane was prepared by sonication following the method of Lien [19]. The activity of photosystem I (PS I) was determined in the presence of 3, 4-dichlorophenyl 1,1-dimethlylurea (DCMU) by recording the O_2 consumption after the addition of ascorbate, 2,6-dichlorophenol indophenol (DCPIP), and methyl viologen (MV). Likewise, the photosystem II (PS II) activity was measured in terms of the O_2 evolution in the presence of p-benzoquinone (PBQ). The redox coupling activity between the two photosystems was measured in the presence of potassium ferricyanide ($K_3Fe[CN]_6$) as an electron-acceptor and H_2O as the electron donor. The complete reactions can be expressed as follows:

Reaction 1. Ascorbate $\cdots\rightarrow$ DCPIP MV $\cdots\rightarrow$ (PS I)

Reaction 2. $H_2O \cdots\rightarrow$ PBQ (PS II)

Reaction 3. $H_2O \cdots\rightarrow$ Ferricyanide (redox coupling between two photosystems).

ATP Content

The size of the ATP pool was measured as per the methods of Larsson and Olsson [18]. Control and treated cells

incubated for known time intervals were withdrawn, centrifuged, and the pellet treated with TCA. The sample was diluted with a Tris buffer to a final TCA concentration of <0.1%. The ATP content was then measured by a luciferin-luciferase assay using an LKB-1250 luminometer.

CO_2 Fixation

The $^{14}CO_2$ uptake by the control and treated cells was measured according to the method of Rai and Raizada [26]. A 0.2 ml sodium bicarbonate ($NaH^{14}CO_3$) solution was added to a 1-ml algal culture and kept in the light for 2 h. The reaction was stopped by adding 0.2 ml of 50% acetic acid and bubbling with air for 5 min. This was then followed by the addition of 5 ml of a scintillation cocktail. The activity was measured in an LKB 1209 Rackbeta Liquid Scintillation counter and expressed in cpm.

Lipid Peroxidation

The peroxidation of lipids by the cells treated with a pesticide and UV-B individually as well as in combination was measured according to the TBA-rm (thiobarbuteric acid-reactive material) method of De Vos *et al.* [7]. The values were presented as the differences between the peroxidized lipids in the control and in the treated cells.

Membrane Permeability

A 5-ml algal culture (control and treated) was centrifuged and concentrated to 3 ml, then heated in a water bath up to 15 min and centrifuged. The supernatant was used to measure the loss of potassium and sodium from the cells following the method of De Filippis [6], using an Atomic Absorption Spectrophotometer.

Nitrogenase Activity (N_2ase)

The *in vivo* nitrogenase activity of the test cyanobacterium was determined by an acetylene-ethylene assay [37]. Two ml of treated cells was taken in 8-ml serum vials and 10% C_2H_2 gas introduced in all the tubes. The vials were incubated in the light for 2 h. The reaction was then stopped by adding 50% acetic acid. One-half ml of the gaseous phase was taken and analyzed in a gas liquid chromatograph (5700 Nucon Gas Chromatograph; Nucon Engineers, New Delhi, India) equipped with a Porapack R column and flame ionization detector.

RESULTS

Survival and Growth Behavior

Approximately 25 and 50% survival of the test cyanobacterium was noticed after 12 and 26 min of UV-B exposure, respectively. However, in the case of the pesticides, the LC_{25} and LC_{50} concentrations were 120 and 300 ppm for carbofuran and 4 and 10 ppm for butachlor, respectively.

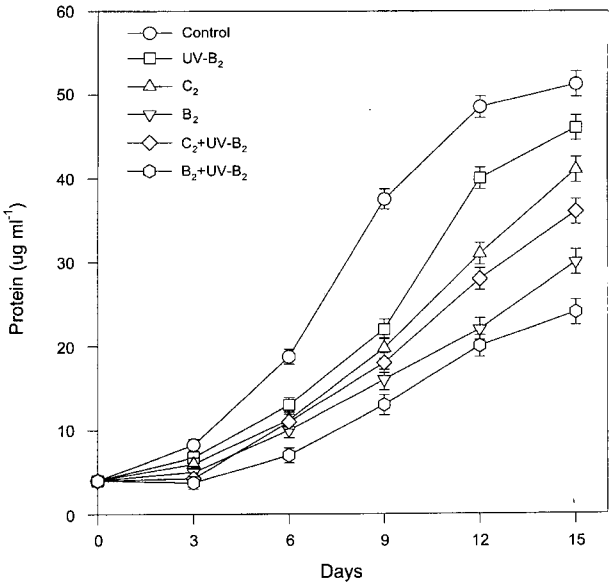

Fig. 1. Effect of UV-B and pesticides on growth performance of *Anabaena doliolum*.
Symbols B_2, C_2, UV-B_1, and UV-B_2 are the same as in Table 1.

Figure 1 shows a general inhibition of growth due to the stresses used in the current study. Approximately 10, 27, and 20% reduction in the protein content of the cyanobacterium was noticed at an LC_{50} concentration of UV-B, butachlor, and carbofuran, respectively. Yet, the inhibition of the protein content reached up to 54 and 41%, when UV-B interacted with butachlor and carbofuran, respectively.

Photosynthesis

Tables 1 and 2 present data on the O_2 evolution, electron transport chain activity, ATP content, and carbon fixation of *Anabaena doliolum* as influenced by UV-B individually as well as in conjunction with butachlor and carbofuran. The photosynthetic O_2 evolution was reduced up to 35 and 49% after 12 and 26 min exposure to UV-B, respectively. Similarly, a reduction of about 42 and 55% was observed in the case of butachlor and 35 and 45% in the case of carbofuran (Table 1) with LC_{25} and LC_{50} doses, respectively. ANOVA indicated that the inhibition of O_2 evolution by UV-B and pesticides was significant ($P<0.01$). The above table also showed that the LC_{25} doses of UV-B and pesticides were more toxic to O_2 evolution than the other parameters investigated. The reduction of O_2-evolution was higher in the case of butachlor than carbofuran (Table1).

All the components of the electron transport chain, i.e. PS II, whole chain, and PS I, showed varying degrees of responses to the stress factors. An appreciable inhibition of O_2 evolution was noticed, when the UV-B and pesticides were combined. However, the inhibition was less than the additive value. A 26 min (LC_{50}) exposure of PS II and the

Table 1. Interactive effects of UV-B, butachlor, and carbofuran on photosynthesis, photosynthetic electron transport chain, and ATP content of *Anabaena doliolum*.

Treatment	O_2 evolution (μmol O_2 evolved/ μg protein/h)	PS I activity (μmol O_2 consumed/ μg protein/h)	PS II activity (μmol O_2 evolved/ μg protein/h)	Whole chain (μmol O_2 evolved/ μg protein/hr)	ATP pool (μmol ATP/ μg protein)	$^{14}CO_2$ fixation (cpm×10^3)
Control	15.8±0.4	135.4±0.3	52.03±0.3	53.66±0.2	0.17±0.2	14.92±0.50
UV_1	10.18±0.2 (35.6)	127.3±0.6 (5.94)	44.33±0.4 (17.77)	48.08±0.3 (10.4)	0.141±0.4 (17.54)	11.89±0.6 (20.31)
UV_2	8.10±0.3 (48.73)	117.6±0.5 (13.15)	39.49±0.3 (24.07)	44.85±0.4 (16.5)	0.058±0.5 (66.08)	9.95±0.7 (33.31)
B_1	9.06±0.2 (42.66)	123.8±0.4 (8.57)	39.49±0.3 (24.07)	46.45±0.3 (16.34)	0.132±0.1 (22.81)	11.58±0.6 (22.39)
B_2	7.04±0.4 (55.44)	100.36±0.5 (25.88)	26.26±0.4 (47.5)	33.3±0.4 (37.94)	0.042±0.2 (75.44)	9.19±0.6 (38.41)
C_1	10.27±0.3 (35.01)	124.8±0.3 (7.82)	39.99±0.3 (23.14)	43.37±0.4 (19.17)	0.137±0.01 (19.23)	11.03±0.5 (26.09)
C_2	8.62±10.4 (45.44)	103.17±0.4 (23.8)	26.93±0.3 (48.25)	31.69±0.3 (40.94)	0.055±0.05 (67.69)	8.44±0.6 (43.41)
UV_1B_1	6.28±0.4 (60.25)	86.9±0.4 (35.88)	36.71±0.4 (29.42)	30.61±0.5 (42.96)	0.104±0.1 (39.18)	9.46±0.7 (36.5)
UV_2B_1	6.0±0.4 (62.03)	72.14±0.5 (46.7)	29.32±0.3 (43.63)	27.41±0.4 (48.92)	0.084±0.2 (50.88)	8.56±0.6 (42.64)
UV_1B_2	5.21±0.3 (67.03)	58.53±0.3 (56.77)	22.90±0.4 (55.97)	22.48±10.3 (58.11)	0.073±0.5 (57.31)	7.06±0.5 (52.82)
UV_2B_2	4.84±0.4 (69.37)	50.4±0.4 (62.73)	17.50±0.5 (66.35)	16.10±0.4 (70)	0.044±0.05 (74.27)	4.19±1.6 (71.92)
UV_1C_1	8.35±0.2 (47.16)	94.54±0.3 (30.18)	40.56±0.3 (22.04)	34.09±0.2 (36.47)	0.123±0.07 (27.7)	9.26±0.5 (37.96)
UV_2C_1	8.27±0.3 (47.63)	73.00±0.3 (45.72)	31.76±0.3 (38.96)	30.76±0.3 (42.67)	0.105±0.02 (38.46)	8.13±0.6 (45.52)
UV_1C_2	8.23±0.2 (47.89)	67.84±0.4 (49.89)	25.15±0.4 (51.66)	24.36±0.4 (54.6)	0.098±0.03 (42.31)	7.35±0.5 (50.73)
UV_2C_2	6.77±0.4 (57.23)	45.55±0.3 (66.36)	20.28±0.3 (61.03)	17.57±0.4 (67.25)	0.054±0.01 (68.46)	5.63±0.6 (62.23)

All the values are ±S.D. Data in parentheses show percentage inhibition. ANOVA significant at $P<0.01$. Control: cells grown under visible light. UV-B_1 and UV-B_2 correspond to 12 and 26 min exposure, respectively, of UV-B radiation at a dose of 12.9 m Wm^{-2} nm^{-1}. B_1 and B_2 are LC_{25} and LC_{50} concentrations of butachlor respectively. C_1 and C_2 are LC_{25} and LC_{50} concentrations of carbofuran, respectively.

whole chain to UV-B caused a 25 and 16% reduction in O_2-evolution, respectively, while 13% less O_2 was consumed in PS I. Similarly, the O_2-evolution was decreased by 47 and 38 and 48 and 41% in the case of PS II and the whole chain at LC_{50} doses of butachlor and carbofuran, respectively. The O_2 consumption by PS I was the same for both butachlor and carbofuran. The combination of UV_2 with butachlor and carbofuran produced a 66 and 61% inhibition of O_2-evolution in PS II and 70 and 67% inhibition of the whole chain electron transport, while the O_2 consumed in PS I was 62.7 and 66%, respectively. The inhibition produced by the UV-B and pesticides individually as well as in combination was significant ($P<0.01$). It is also pertinent to mention that the whole chain electron transport was more sensitive to the UV-B and pesticide interaction than PS I and PS II; the UV_2+B_2 combination depicted a synergistic inhibition of the whole chain electron transport.

Likewise, the $^{14}CO_2$ fixation was reduced by 20 and 34% after 12 and 26 min of UV-B exposure, respectively. The inhibition ($P<0.01$) was 22 and 38% for butachlor, and 26 and 43% for carbofuran (Table1) at LC_{25} and LC_{50} doses, respectively. Table 1 further reveals that the inhibition of $^{14}CO_2$ fixation by the UV-B and pesticides was less than the additive effect. The individual effect of the two pesticides was greater than the UV-B effect. Both pesticides showed an appreciable inhibition of the $^{14}CO_2$ uptake while interacting with UV-B; the inhibition produced by UVB_2 together with LC_{50} doses of the two pesticides was additive.

The inhibition of the ATP content did not violate the general trend of dose-dependent inhibition. However, one interesting point emerged from the ATP data was that an LC_{50} dose of UV-B and pesticides was exceedingly toxic to this parameter. The inhibition generated by the combination of the two stresses did not even reach the level of inhibition produced by the stresses, when used individually. The ATP

Table 2. Interactive effects of UV-B+butachlor and UV-B+carbofuran on loss of Na^+ and K^+ and lipid peroxidation of *A. doliolum*.

Treatment	Butachlor			Carbofuran		
	Na^+ efflux (μm Na^+/ μg protein)	K^+ (μm K^+/ μg protein)	Lipid peroxidation TBA-rm ($A_{532-600}$/mg protein)	Na^+ efflux (μm Na^+/ μg protein)	K^+ (μm K^+/ μg protein)	Lipid peroxidation TBA rm ($A_{532-600}$/mg protein)
Control	–	–	0.29±0.2	–	–	0.23±0.03
UVB_1	1.2±0.01 (19.7)	1.05±0.02 (30)	0.36±0.03	1.2±0.01 (29.4)	1.05±0.02 (30)	0.28±0.04
UVB_2	2.28±0.02 (37.4)	1.42±0.01 (41)	0.59±0.03	2.30±0.02 (37.09)	1.40±0.03 (40)	0.63±0.04
P_1	1.74±0.03 (28.52)	1.32±0.02 (38)	0.52±0.04	1.61±0.01 (25.97)	1.23±0.05 (35)	0.39±0.03
P_2	2.52±0.01 (41.31)	1.65±0.02 (48)	0.90±0.06	2.39±0.02 (38.6)	1.54±0.02 (44)	0.75±0.05
UV_1P_1	2.16±0.01 (36)	1.58±0.02 (456.5)	0.69±0.05	1.97±0.01 (31.8)	1.33±0.4 (38)	0.45±0.04
UV_2P_1	2.80±0.02 (47)	2.03±0.03 (59.7)	0.70±0.03	2.50±0.01 (40.32)	1.79±0.03 (51)	0.49±0.05
UV_1P_2	3.18±0.01 (53)	2.28±0.02 (67.05)	0.85±0.04	3.04±0.02 (49.03)	2.11±0.02 (60.28)	0.63±0.03
UV_2P_2	3.48±0.02 (58)	2.70±0.03 (79.4)	1.28±0.06	3.30±0.01 (53.22)	2.43±0.03 (69.43)	0.88±0.03

All the values are ±S.D. Data in parentheses show percentage inhibition. ANOVA significant at $P<0.01$. Control: cells grown under visible light. P_1, P_2 are LC_{25}, LC_{50} concentrations, respectively, of the pesticides used. UV-B_1 and UV-B_2 correspond to 12 and 26 min exposure, respectively, of UV-B radiation at a dose of 12.9 m Wm^{-2} nm^{-1}.

content was inhibited by 66% after 26 min of UV-B exposure. The inhibition was 75% for butachlor and 67% for carbofuran (Table 1) with LC_{50} doses. This table also shows that the ATP content was significantly inhibited by UV_2P_2 ($P<0.01$).

Lipid Peroxidation

Table 2 shows a significant ($P<0.01$) inhibitory effect of UV-B and the pesticides on lipid peroxidation. Approximately 2.7-fold increase in lipid peroxidation, i.e., from 0.29 to 0.78 TBA-rm after 26 minutes of UV-B exposure, was observed. However, with LC_{50} doses of butachlor and carbofuran, there was a 3.1- and 3.26-fold increase in lipid peroxidation, respectively. In the case of UVB_2P_2 (butachlor), the lipid peroxidation was 4.9 times that of the control and it was 3.8 times for carbofuran. It is also worth mentioning that the stress factors in combination induced significant lipid peroxidation, yet this was less than their additive effect. As such, the effect of treatment and the dosage of the stress factors on lipid peroxidation was highly significant ($P<0.01$).

Membrane Permeability

Table 2 shows that the exposure of the cyanobacterium to LC_{25} and LC_{50} doses of UV-B and pesticides (butachlor and carbofuran) generated an Na^+ efflux of 20.29, and 27% and 38.42, and 40%, respectively. Similarly, there was an K^+ efflux of 30.38 and 35%, and 40.47 and 44%, respectively, at the above doses of the stress factors. The loss of K^+ was always higher than that of Na^+. As such, neither pesticide produced an appreciable difference in the membrane permeability, as expressed in terms of the loss of Na^+ and K^+.

Nitrogenase Activity (N_2ase)

The exposure of the cyanobacterium to LC_{25} and LC_{50} doses of UV-B, butachlor, and carbofuran decreased the nitrogenase activity from 6.5 μM C_2H_4 μg $protein^{-1}$ h^{-1} to 5.19 and 4.16, 4.34 and 3.25, and 4.73 and 3.76 μM C_2H_4 μg $protein^{-1}$ h^{-1}, respectively (Fig. 2). Therefore, this figure

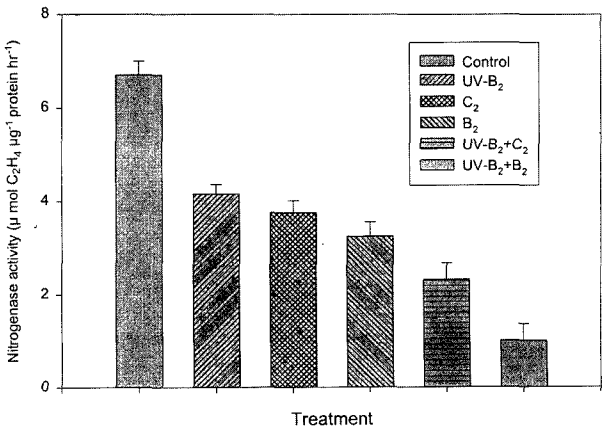

Fig. 2. Interactive effect of UV-B and pesticides on the nitrogenase activity of *Anabaena doliolum*.
Symbols B_2, C_2, and UV-B_2 are the same as in Table 1.

shows a dose-dependent, highly significant inhibition of nitrogenase activity ($P<0.01$). Both pesticides appreciably inhibited the N_2ase activity, which was more than that produced by UV-B. Furthermore, the damage caused by butachlor was more pronounced than that caused by carbofuran.

DISCUSSION

The results of the present study vividly demonstrated that *Anabaena doliolum* is highly sensitive to UV-B and pesticides. The parameters investigated were significantly inhibited by the stress factors ($P<0.01$). The growth inhibition (Fig. 1) of the cyanobacterium might have been due to the inhibition of the cell division, cellular constituents, and physiological processes [3, 31]. The reduction in the protein content (Fig. 1) by UV-B might have been due to the denaturation of proteins following absorption of UV-B (290–315 nm) by aromatic amino acids present in the proteins. Furthermore, UV-B irradiation is known to cause a number of modifications in proteins such as photo-degradation, an increased aqueous solubility of the membrane proteins, and fragmentation of the peptide chains, leading to the inactivation and disruption of their structural sub-units [13]. The loss of structure and function of proteins due to UV-B irradiation can lead to changes in the cell membrane permeability, resulting in the ultimate death of the organism [34].

The UV-B and pesticide-induced reduction in photosynthetic O_2 production might have been due to the inhibition of PS II activity as a result of damage of the thylakoid lamellar membrane [27], disruption of the water splitting machinery of the photosynthetic apparatus, or damage of the reaction center of PS II [23, 33]. The above arguments were also supported by a significant correlation between the O_2 evolution and the PS II activity ($r=0.83$; $P<0.001$).

The significant reduction in the PS II and whole chain activity produced by UV-B together with butachlor and carbofuran (Table 1) might have been due to the damage of PS II [23, 28]. The increased inhibition of the whole chain activity compared to PS II may have been due to UV-B induced damage of the intrinsic membrane proteins and electron carriers between PS II and PS I [25]. Pesticides are also known to affect the PS II and electron transfer from Q^- to plastoquinone [5, 28]. The increased inhibition of the whole chain by UV-B and the pesticides might have been due to the binding of the latter to certain proteinaceous molecules located on the surface of the photosynthetic membranes [20].

Furthermore, the decreased ATP content (Table 1) seemed to be the primary cause for the inhibition of the electron transport system [27]. A significant positive correlation between the ATP pool and the inhibition of the whole chain electron transport ($r=0.68$; $P<0.001$) also offers support to the above supposition.

The significant inhibition of the ^{14}C uptake by UV-B and the pesticides (butachlor and carbofuran, Table 1) might have been due to the damage of the photosynthetic apparatus, resulting in a reduced supply of ATP and $NADPH_2$ [8]. Pesticides are proven inhibitors of ^{14}C uptake, the electron transport chain, and O_2 evolution [14, 28]. This dependence of carbon fixation on ATP was also supported by a highly positive correlation between the ATP pool and the ^{14}C uptake ($r=0.80$; $P<0.001$).

The UV-B and pesticide-induced peroxidation of lipids was elegantly demonstrated by a positive correlation with the dosage of the stress factors ($r=0.32$; $P<0.001$). The peroxidation of lipids in many biological systems including algae would appear to be due to the energetic cleavage of covalent bonds by UV-B-generated amino acid radicals that can oxidize fatty acids [32] or through the activation of toxic O_2 molecules that can attack fatty acid chain through disruption by UV-B and pesticides [17, 22].The current results of lipid peroxidation (Table 2) suggested that UV-B and the pesticides affected the permeability of the plasma membrane through peroxidation of the membrane lipids, thereby facilitating Na^+ and K^+ leakage. This was supported by a positive correlation ($r=0.23$) between K^+ efflux and lipid peroxidation.

An appreciable reduction in nitrogenase activity by UV-B and the pesticides ($P<0.01$) might have been due to an interruption in the supply of ATP and reductants to the enzyme for its activity. This was also supported by a highly significant correlation between the ATP pool and nitrogenase activity ($r=0.78$; $P<0.001$), as reported earlier [16, 33, 35]. The interaction of UV-B and the pesticides also significantly suppressed the nitrogenase activity ($P<0.01$). Accordingly, the current study showed that the whole chain electron transport was highly sensitive to the interactive effects of UV-B and pesticides, yet still less than the additive effect on the different metabolic processes. The pesticides used in the present study were expected to be more toxic while interacting with UV-B. However, such an effect was not observed and the effect of their interaction was still less than the additive effect. This might be due to a hitherto unknown photodegradation of the pesticides by UV-B radiation in the aqueous solution.

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REFERENCES

1. Adhikary, S. P., P. Dash, and H. Pattnaik. 1984. Effect of the carbamate insecticide sevin on *Anabaena* sp. and *Westiellopsis prolifica*. *Acta. Microb. Hung.* **31:** 335–338.
2. Arnotts, T. and T. M. Murphy. 1991. A comparison of a fungal elicitor and ultraviolet radiation on ion transport and hydrogen peroxide synthesis in rose cells. *Environ. Exp. Bot.* **31:** 209–216.
3. Behrenfeld, M. J., II H. Lee, and L. F. Small. 1994. Interactions between nutritional status and long term responses to UV-B radiation stress in a marine diatom. *Mar. Biol.* **118:** 523–530.
4. Bornman, J. F. 1989. Target site of UVB radiation in photosynthesis of higher plants. *J. Photochem. Photobiol.* **4:** 145–158.
5. Chaturvedi, R. and R. Shyam. 2000. Degradation and de novo synthesis of D1 protein and psb A transcript levels in *Chlamydomonas reinhardtii* during UV-B inactivation of photosynthesis and its reactivation. *J. Biosci.* **25:** 65–71
6. De Filippis, L. F. 1979. The effect of heavy metal compounds on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol.* **92:** 39–49.
7. De Vos, C. H. R., H. Schat, R. Vooijs, and W. H. O. Ernst. 1989. Copper induced damage to the permeability barrier in roots of *Silene cucubalus*. *J. Plant Physiol.* **135:** 164–169.
8. Döhler, G., I. Biermann, and J. Zink. 1986. Impact of UV-B radiation on photosynthetic assimilation of ^{14}C-bicarbonate and inorganic ^{15}N-compounds by cyanobacteria. *Z. Naturforsch.* **41:** 426–432.
9. El-Dib, M. A., Salwa A. Shehata, and Hoda F. Abou-Waly. 1991. Response of freshwater algae (*Scenedesmus* spp.) to phenylurea herbicides. *Water, Air & Soil Pollution* **55:** 295–303.
10. Gerloff, G. C., G. P. Fitzerald, and F. Skoog. 1950. The isolation, purification and culture of blue-green algae. *Am. J. Bot.* **27:** 216–218.
11. Graham, J. C., C. Underwood, K. S. Nilsson, and W. Angela. 1999. Short-term effects of UVB radiation on chlorophyll fluorescence, biomass, pigments and carbohydrate fractions in a benthic diatom mats. *J. Phycol.* **35:** 656–666.
12. Häder, D. P. 1997. Effects of UV radiation on phytoplankton. *Adv. Microb. Ecol.* **15:** 1–26.
13. Häder, D. P. 2001. Adaptation to UV stress in algae, pp. 173–202. *In* Rai, L. C. and Gaur, J. P. (eds.) *Algal Adaptation to Environmental Stresses: Physiological, Biochemical and Molecular Mechanisms.* Springer, Heidelberg, Germany.
14. Heckman, C. W. 1994. Pesticide chemistry and toxicity to algae. *In* Rai, L. C., Gaur, J. P., and Soeder, C. J. (eds.) *Algae and Water Pollution. Adv. Limnol.* **42:** 205–234.
15. Jordan, B. R. 1993. The molecular biology of plants exposed to ultraviolet B radiation and the interactions of other environmental stresses, pp. 153–170. *In* Jackson, M. B. (ed.) *Interacting Stresses on Plants in a Changing Climate.* NATO ASI Series.
16. Kashyap, A. K. and K. D. Pandey. 1982. Inhibitory effects of rice field herbicide machete on *Anaebaena doliolum* Bharadwaja and protection by nitrogen sources. *Z. Pflanzen. Physiol.* **107:** 339–345.
17. Lal, R. and D. M. Saxena. 1980. Cytological and biochemical effects of pesticides on microorganisms. *Residue Rev.* **73:** 49–86.
18. Larson, C. M. and T. Olsson. 1979. Firefly assay of adenine nucleotide from algae: Comparison of extraction methods. *Plant Cell Physiol.* **22:** 145–155.
19. Lien, S. 1978. Hill reaction and phosphorylation with chloroplast preparation from *Chlamydomonas reinhardtii*, pp. 305–315. *In* Hellebust, J. A. and Craigie, J. S. (eds.) *Handbook of Phycological Methods: Physiological and Biochemical Methods*, Cambridge University Press, U.K.
20. Lohmann, E. and H. Hagedorn. 1986. The effect of parathion on green algae. The effect on photosynthesis. *Appl. Microbiol. Biotechnol.* **23:** 507–509.
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 269–275.
22. Malanga, G. and S. Punturalo. 1995. Oxidative stress and antioxidant content in *Chlorella vulgaris* after exposure to ultraviolet-B radiation. *Physiol. Plant* **94:** 672–679.
23. Murthy, S. D. S. and S. Rajagopal. 1995. UV-B radiation induced alterations in the bioenergetic process of photosynthesis. *Photosynthetica* **31:** 481–487.
24. Noorudeen, A. M. and G. Kulandaivelu. 1982. On the possible site of inhibition of photosynthetic electron transport by ultraviolet B (UVB) radiation. *Physiol. Plant* **55:** 161–166.
25. Oscar, S., M. A. K. Bernd, and B. P. Barbara. 1995. Impact of ultraviolet B radiation on photosystem 2 activity and its relationship to the inhibition of carbon fixation rates for Antarctic ice algae communities. *J. Phycol.* **31:** 703–715.
26. Rai, L. C. and M. Raizada. 1985. Effect of nickel and silver ions on survival, growth carbon fixation and nitrogenase activity of *Nostoc muscorum*: Regulation of toxicity by EDTA and calcium. *J. Gen. Appl. Microbiol.* **31:** 329–337.
27. Rai, L. C., B. Tyagi, and N. Mallick. 1996. Alteration in photosynthetic characteristics of *Anabaena doliolum* following exposure to UV-B and Pb. *Photochem. Photobiol.* **64:** 658–663.
28. Ralph, P. J. 2000. Herbicide toxicity of *Halophila ovalis* assessed by chlorophyll *a* fluorescence. *Aquat. Bot.* **66:** 141–152
29. Rath, B. and S. P. Adhikary. 1994. Relative tolerance of several nitrogen fixing cyanobacteria to commercial grade furadan (carbofuran 3%). *Ind. J. Expt. Biol.* **32:** 213–215.
30. Rath, B. and S. P. Adhikary. 1996. Effects of pH, irradiance and population size on the toxicity of furadan to two species of *Anabaena*. *Biologia Plantarum* **38:** 402–404.
31. Ravindran, M., C. R. Sugnna, and S. Shanmugasundarum. 2000. Tolerance of *Oscillatoria* isolates to agrochemicals and pyrethroid components. *Ind. J. Expt. Biol.* **38:** 402–404.
32. Salmon, S., J. C. Maziere, R. Santus, P. Morliere, and N. Bouchemal. 1990. UV-B induced photoperoxidation of lipids of human low and high density lipoprotein. A possible

role of tryptophan residues. *Photochem. Photobiol.* **52:** 541–545.
33. Singh, L. J. and D. N. Tiwari. 1988. Effects of selected rice field herbicides on photosynthesis, respiration and nitrogen assimilating enzyme systems of paddy soil diazotrophic cyanobacteria. *Pestic. Biochem. Physiol.* **31:** 120–128.
34. Sinha, R. P. and D. P. Häder. 1998. Effects of ultraviolet B radiation in three rice field cyanobacteria. *J. Plant Physiol.* **153:** 763–769.
35. Sinha, R. P., N. Singh, A. Kumar, H. D. Kumar, M. Häder, and D. P. Häder. 1996. Effects of UV irradiation on certain physiological and biochemical processes in cyanobacteria. *J. Photochem. Photobiol. B: Biol.* **32:** 107–113.
36. Smith, R. C., K. S. Baker, O. H. Hansen, and R. Olson. 1980. Photoinhibition of photosynthesis in natural water. *Photochem. Photobiol.* **31:** 585–592.
37. Stewart, W. D. P., G. P. Fitzgerald, and R. H. Burris. 1968. Acetylene reduction by nitrogen fixing blue-green algae. *Arch. Microbiol.* **62:** 336–348.
38. Wilson, M. I. and B. M. Greenberg. 1993. Protection of the D1 photosystem II reaction centre protein from degradation in ultraviolet radiation following adaptation of *Brassica napus* L to growth in ultraviolet B. *Photochem. Photobiol.* **57:** 556–563.