Research Article

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Insecticide induced enhanced effect of UV-B on growth and photosynthesis of cyanobacterium *Nostoc muscorum*

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Cyanobacterium *Nostoc muscorum* was used in the present study to evaluate the effect of UV-B irradiation (5 min and 15 min) and monocrotophos (10 ppm) on the survival, growth, photosynthetic pigment content, photosynthetic oxygen evolution and ^{14}C-fixation, either alone or in combination. Both the stresses caused reduction in survival, growth and photosynthetic pigments. Among photosynthetic pigments phycocyanin was the main target to UV-B and monocrotophos followed by chlorophyll *a* and carotenoids. The strong inhibition on photosynthetic oxygen evolution and ^{14}C-fixation was noticed on exposing the culture to UV-B and monocrotophos. However, the impact on ^{14}C-fixation was higher as compared to the rate of oxygen evolution. The present findings suggest that the damage caused by individual stress may enhance in the presence of multiple stresses.

Key words: Growth, Insecticide, Monocrotophos, Photosynthetic oxygen evolution, Pigments, Ultraviolet-B

The application of pesticides, such as insecticides, in agriculture to reduce or destroy pests in the modern age has led to serious contamination of aquatic ecosystems resulting in greater damage to wide variety of beneficial microorganisms (Dubois *et al.*, 1996; Shetty *et al.,* 2000). Alteration of the species composition of an aquatic community as a result of toxic stress may affect the structure and the functioning of the whole ecosystem (Campanella *et al.,* 2000; Wong, 2000). Water bodies such as paddy fields, aquaculture and ponds are highly eutrophic and maintained large standing crops of phytoplankton, particularly cyanobacteria (Millie *et al.,* 1992). Cyanobacteria harvest light energy during the process of photosynthesis and assimilate it into carbon compounds, which provide cellular energy and also carbon skeleton for metabolic process such as nitrogen fixation (Prasad *et al.,* 2005). Because of the dual characteristics features cyanobacteria occupy an important place in both aquatic and terrestrial ecosystems (Venkataraman, 1981). Therefore, any adverse effect of insecticides may severely affect photosynthesis and related metabolic processes and overall growth performance of cyanobacteria, and finally altered the species composition in these ecosystems. A significant of information on the toxicological aspects of pesticides on plants and algae are available (Prasad and Zeeshan, 2005). However, little about the toxicological aspects of insecticides on cyanobacteria is known (Abou-waly *et al.,* 1991; Sabater and Carraso, 2001). Besides pesticides, cyanobacteria may also experience many kinds of stresses such as metal, salinity and UV-B etc. in the natural environment. UV-B irradiation has been demonstrated to cause damaging effects on nucleic acids, proteins and lipids and thus, it produces depression of key physiological processes (Teramura and Sullivan, 1994; Jansen *et al.,* 1998; Hideg *et al.,* 2006).UV-B induced adverse impact on whole cell photosynthetic oxygen evolution, carbon fixation and photosynthetic electron transport activity of *Plectonema boryanum* was observed in earlier findings (Prasad and Zeeshan, 2004).

In recent years it has been demonstrated that the effectiveness of UV-B irradiation on

growth and certain metabolic activities of plants may get altered due to other stresses such as ozone (Rao *et al.,* 1996), heavy metals (Dube and Bornman, 1992) etc. Recently, the combined effect of UV-B and metal on growth, nutrient uptake and photosynthesis in *Anabaena doliolum* was studied (Rai *et al.,* 1998). To our knowledge no attempts has been made to find out the response of growth and photosynthesis of *Nostoc muscorum* due to simultaneous exposure to both insecticide monocrotophos and UV-B irradiation, a situation likely to exist in the natural environment. Therefore, we tested the impact of UV-B and insecticide monocrotophos either alone or in combination on growth, photosynthetic pigments, whole cell photosynthetic oxygen evolution and carbon fixation rate in cyanobacterium *N. muscorum.*

MATERIAL AND METHODS

Organism and culture conditions: The filamentous, heterocystous, cyanobacterium *Nostoc muscorum* was isolated from rice fields near Allahabad, India and raised to axenic culture. The culture was grown in nitrogen free Chu-10 medium (pH 7.5) and maintained in the culture room at 25 ± 2 °C under 75 μmol m^{-2} s^{-1} photon flux density (PFD) with a photoperiod of 14:10 h.

UV-B and monocrotophos treatment: The homogenized culture suspension of *Nostoc muscorum* (dry weight, 0.1 mg ml^{-1}) from exponential phase was taken in sterilized 7.5 cm diameter Petri- dishes and exposed to artificial UV-B irradiation along with cool white fluorescent light of 20 μmol m^{-2} s^{-1} PAR intensity. The source of UV-B radiation was single UV-Lamp (TL 40 W/12 Philips, Holland) with its main output at 312 nm. Radiation dose of 5 and 15 min UV-B exposure at the surface of culture were 0.12 and 0.36 kJ m^{-2}, respectively. Culture suspension preincubated with 10 ppm monocrotophos for 12 h was exposed to selected dose of UV-B to evaluate the interactive effects of these stresses. The irradiance was measured with the help of power meter (Spectra Physics, USA model 407, A-2). For the preparation of stock solution monocrotophos was prepared in a little amount of 70% ethanol and the solution was further sterilized by passing through a millipore membrane filter (0.22 μm). From this stock solution, required concentration of monocrotophos was prepared in nutrient medium.

Measurement of growth and photosynthetic pigments: Growth was determined after four days of treatments by estimating protein content as per the method of Lowry *et al.* (1951). Chlorophyll *a* and carotenoids were extracted in 80 % acetone and measured according to Myers and Kratz (1955). Phycocyanin was extracted in 2.5 mM potassium phosphate buffer (pH 7.0) after repeated freezing and thawing, and absorbance was measured at 620 nm as per the method of Blumwald and Tel-Or (1982).

Photosynthetic O_2 evolution: Whole cell O_2 evolution of treated and untreated cells was measured with a Clark type O_2 electrode (Rank Brothers, UK) in a temperature controlled airtight reaction vessel at 28 ^oC. The cyanobacterial cells were illuminated by a projector lamp provided with 360 μmol m^{-2} s^{-1} PAR photon flux density. The photosynthetic rate was expressed as μmol O_2 evolved mg^{-1} protein h^{-1}.

^{14}C- fixation: To study the photofixation of carbon, 0.05 ml of $NaH^{14}CO_3$ (Specific activity 9.25×10^4 Bq ml^{-1}) was added to 5 ml of treated and untreated culture suspension and incubated for 10 min under light with 360 μmol photon m^{-2} s^{-1} PAR photon flux density. The reaction was terminated by addition of 0.1 ml of 2N HCl and the samples were flushed with air for 30 min to remove the dissolved $^{14}CO_2$.The radioactivity of ^{14}C-fixed in photosynthates was counted by LKB-1209 Rack Beta liquid scintillation counter. Rate of ^{14}C-fixation is expressed as count per minute [CPM (μg Chl *a*)$^{-1}$ h^{-1} x 10^2].

RESULTS AND DISCUSSION

The survival of the *N. muscorum* based on colony count method was recorded following UV-B and monocrotophos either alone or in combination (Fig. 1). UV-B dose for 5 min and 15 min declined the survival by 10 % and 20 %, respectively. The selected dose of monocrotophos (10 ppm) reduced the survival by 20 %. The survival declined more rapidly when both the doses of UV-B (5 min and 15 min) were combined with 10 ppm of monocrotophos.

Similar to the survival, the growth of *N. muscorum* in liquid medium was also studied

Table 1. Interactive effect of UV-B and monocrotophos (M) on growth and photosynthetic pigments of *N. muscorum*.

Treatment	Protein (μg ml^{-1})	Chl *a* (μg ml^{-1})	Car (μg ml^{-1})	Phy (μg ml^{-1})
Control	65 ± 1.0	1.52 ± 0.02	0.59 ± 0.01	18.24 ± 0.4
UV-B_5	56 ± 0.9 (14)	1.42 ± 0.02 (6)*	0.56 ± 0.01 (4)*	16.96 ± 0.1 (7)*
UV-B_{15}	48 ± 0.8 (26)	1.30 ± 0.01 (14)	0.53 ± 0.02 (9)*	13.86 ± 0.4 (24)
M_{10}	45 ± 0.5 (31)	1.14 ± 0.02 (25)	0.45 ± 0.01 (23)	12.95 ± 0.3 (29)
UV-B_5 + M_{10}	30 ± 0.4 (54)	0.82 ± 0.01 (46)	0.31 ± 0.01 (47)	6.56 ± 0.1 (64)
UV-B_{15} + M_{10}	22 ± 0.2 (66)	0.69 ± 0.02 (54)	0.28 ± 0.01 (52)	5.10 ± 0.2 (72)

Means ± SE, values in parentheses are [%]. All treatment are significantly different ($P < 0.01$) and (*$P < 0.05$) from control (Student's *t*-test).

following 5 and 15 min of UV-B exposure and monocrotophos (M), alone or in combination (Table 1). The inhibitory effect of UV-B and monocrotophos on the growth of cyanobacterium in liquid medium was more pronounced as compared to the survival. Further declined in the growth was noticed following the exposure of combined doses of UV-B and monocrotophos [5 min UV-B + monocrotophos (M_{10}), 15 min UV-B + monocrotophos (M_{10})].

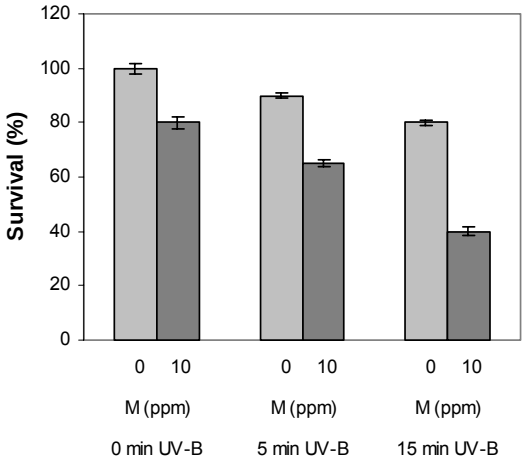

Figure 1. Effects of UV-B and monocrotophos (M), singly and in combination, on survival of *N. muscorum* measured after 15 days of treatment. Mean ± S.E of three replicates.

Both UV-B and monocrotophos reduced the content of Chl *a,* Car and PC, either alone or in combination (Table 1). The decrease in photosynthetic pigments was dose of UV-B dependant. The inhibitory effect of UV-B on pigments became more pronounced when both the doses of UV-B were combined with monocrotophos (M_{10}) separately. Phycocyanin was severely affected as compared to Chl *a* and Car.

The photosynthetic activity *i.e*. whole cell oxygen evolution and $^{14}CO_2$ fixation of *N. muscorum* under UV-B and monocrotophos stress were measured and the results are shown in figures 2 and 3. The rate of O_2 evolution after 4 days of 5 and 15 min UV-B exposure declined by 8 and 18 %, respectively, whereas 10 ppm of monocrotophos treatment exhibited 26 % decline in the activity. The effect of theses stresses was more severe when given together as it decreased by 46 and 58 % with 5 min UV-B + monocrotophos (M_{10}) and 15 min UV-B + monocrotophos (M_{10}), respectively. Similar doses of UV-B and monocrotophos, alone or in combination, caused stronger inhibitory effect on the rate of $^{14}CO_2$ fixation in comparison to the effect on photosynthetic O_2 evolution.

The results of the present study show sensitivity of *Nostoc muscorum* to both UV-B irradiation and insecticide monocrotophos. Survival of the cyanobacterium was affected by both the stresses either alone or in combination (Figure 1). A decrease in survival following UV-B exposure might be due to the damage of cellular constituents, (Caldwell, 1981; Döhler *et al.,* 1986) or metabolic processes (Tyagi *et al.,* 1992) eventually causing death of the cells (Newton *et al.* 1979). Decrease in survival following

monocrotophos treatment indicated that photosynthetic apparatus and pigments synthesis may be the target of this insecticide. Insecticide causes damage to various metabolic processes (Prasad *et al.,* 2005) and chlorophyll *a* synthesis (Kaushik and Venkataraman, 1983).

The inhibition in growth of the cyanobacterium by enhanced UV-B irradiation (Table 1) in the present observation is in agreement with the results of earlier workers (Sinha and Häder, 1998; Prasad and Zeeshan, 2004). The results reveal that increased reduction in the growth of *Nostoc muscorum* following UV-B exposure might be due to reduced photosynthetic activity, nitrogen metabolism and irreparable damage to DNA (Caldwell, 1981; Häder *et al.,* 1986). Monocrotophos induced reduction in the growth of cyanobacterium *Nostoc muscorum* was observed at 10 ppm concentration. Similar inhibitory effect on growth of *Oscillatoria* MKU-123 was also reported by Ravindran *et al.* (2000). It has been suggested by Kaushik and Venkataraman (1983) that reduced growth of cyanobacteria *Westiellopsis, Hapalosiphon* sps and *calothrix* sps was due to the insecticide induced inhibition on photosynthetic activity and nitrogen fixation as these metabolic activities provide precursors for protein and nucleic acid synthesis.

Further, decline in growth of cyanobacterium following UV-B and monocrotophos together was probably due to more severe effects of these stresses on biomolecules and certain metabolic activities.

The reduction in photosynthetic pigments of cyanobacterium *Nostoc muscorum* was noticed following UV-B exposure and monocrotophos (Table 1). The effects was more severe on phycocyanin followed by carotenoids and Chl *a.* Tyagi *et al.,* (1992) reported the damaging effect on photosynthetic pigments and suggested that the reduction in pigment content might have occured due to the bleaching caused by UV-B irradiation. Similar to our results, strong damaging effect on phycocyanin by UV-B has also been observed in earlier findings (Sinha and Häder, 1998). The photosynthetic pigments were also found sensitive to the monocrotophos. These results are in consonance with the deleterious effect of other insecticides on chlorophyll *a*, carotenoids and phycocyanin (Anand and Subramaniam, 1997). Further deterioration in the photosynthetic pigments was noticed when cyanobacterium was exposed to both the stress simultaneously. Strong inhibitory effect of UV-B on phycocyanin could be correlated due to the proteinaceous nature and their localization on the outer surface of thylakoid membrane.

Photosynthetic oxygen evolution in intact cells (Figure 2) and $^{14}CO_2$ fixation (Figure 3) of *N. muscorum* were adversely affected by UV-B irradiation and monocrotophos treatment. Similar effects were noticed in *P. boryanum* following UV-B and monocrotophos treatment (Prasad and Zeeshan, 2004).

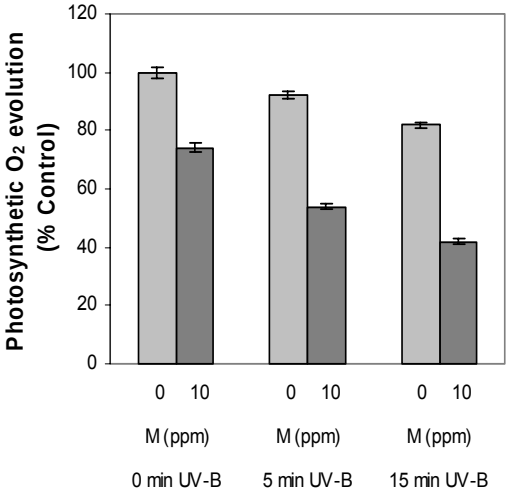

Figure 2. Effects of UV-B and monocrotophos (M), singly and in combination, on photosynthetic O_2 evolution of *N. muscorum*. Photosynthetic O_2 evolution in untreated control was 7.8 ± 0.35 μmol O_2 evolved mg^{-1} protein h^{-1}. Mean ± SE. All the values are significant at $P < 0.01$.

The decrease in the photosynthetic O_2 evolution could be explained on the basis of inhibitory effect of these stresses on PS II activity. Besides this, In the present study the decrease in photosynthetic O_2 evolution may also be correlated with the decrease in contents of photosynthetic pigments (Table 1). The reduced carbon fixation rate in cyanobacterium *N. muscorum* following the treatment of monocrotophos and UV-B probably resulted due to the reduced supply of ATP and NADPH, an assimilatory power for carbon fixation and other metabolic reactions.

It may also be due to the direct effect of UV-B on RUBISCO as noticed in the dinoflagellate *Prorocentrum micans* (Lesser *et al.,* 1994).

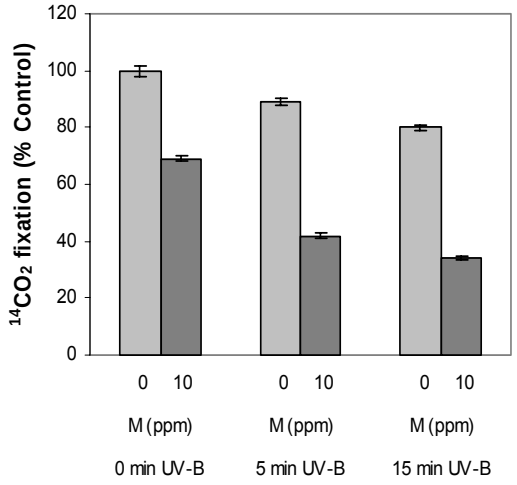

Figure 3. Effects of UV-B and monocrotophos (M), singly and in combination, on ^{14}C-fixation of *N. muscorum*. ^{14}C- fixation in untreated control was 714 ± 27 CPM (μg Chl a)$^{-1}$ h^{-1} x 10^2 Mean ± SE. All the values are significant at *P* < 0.01.

On the basis of present findings it can be concluded that the effect of one stress on survival, growth, photosynthetic pigments and photosynthesis becomes more pronounced in the presence of other stress.

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