

Research Article

Combined Effects of UV Radiation, Photosynthetically Active Radiation and 2,4-Dichlorophenoxyacetic Acid on Pigmentation, Proteins and Antioxidant Enzymes in the Cyanobacterium *Scytonema geitleri* Strain HKAR-12

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Abstract

In this study, we have investigated the combined effects of ultraviolet radiation (UVR) and herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) on chlorophyll a (Chl a), total carotene, protein contents and antioxidative enzymes i.e. superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in the cyanobacterium *Scytonema geitleri* strain HKAR-12. 2,4-D alone stimulated the growth and differentiation of *Scytonema geitleri* strain HKAR-12 in nitrogen free medium at a low concentration 100 µg/mL. While its higher concentration inhibited growth and 500 µg/mL concentration of 2,4-D proved to be lethal. Chl a and protein content were found to be adversely affected by combined exposure of UVR, PAR (Photosynthetically Active Radiation) and 2,4-D after varying duration of treatments. However, a progressive increase in total carotene content was observed upto 48 h of exposure followed by a subsequent decrease. However, synthesis of antioxidative enzymes to counteract the damaging effect of such stressful conditions is one of the several mechanisms adopted by cyanobacteria as their defense strategies. Antioxidative enzymes exhibited differential responses against combined stress of UVR, PAR and 2,4-D. SOD, CAT and APX showed 2-3 fold increase upto 48 h duration of exposure in PAR; PAR + 2,4-D; PAB (PAR + UV-A + UV-B) and PAR + UV-A + UV-B + 2,4-D followed by a gradual decrease in the concentration of these studied enzymes. These results suggest that the combination of UVR and exogenous herbicides such as 2,4-D have detrimental effects on cyanobacterial metabolism.

ABBREVIATIONS

UVR: Ultraviolet Radiation; 2,4-D: 2,4-Dichlorophenoxyacetic Acid; CAT: Catalase; SOD: Superoxide Dismutase; APX: Ascorbate Peroxidase; ROS: Reactive Oxygen Species; PAR: Photosynthetically Active Radiation; PAB: PAR+UV-A+UV-B

INTRODUCTION

Cyanobacteria are Gram-negative, cosmopolitan, photolysis mediated oxygen evolving prokaryotes that can survive and flourish in almost every habitat ranging from hot springs to Arctic and Antarctic regions as well as in the form of symbionts in plants, lichens and several protists [1,2]. They play a significant

role in global photosynthetic biomass production, CO₂ fixation, successional processes, nutrient cycling and as a potent natural biofertilizer in rice paddy fields [3,4].

The depletion of stratospheric ozone has resulted in an increase of deleterious ultraviolet radiation (UVR) at the Earth's surface. These biologically effective doses of UV-B radiation can penetrate deep into the water column, and cause generation of reactive oxygen species (ROS) from the photosynthetic system in plant cells [5]. These increased ROS can easily destroy DNA, proteins and other biological molecules, and subsequently affect growth and reproduction, survival, photosynthetic energy harvesting enzymes, and the content of photosynthetic

pigments [6-9]. However, cyanobacteria have also developed adaptation strategies to counteract the damaging effects of UVR, which include avoidance, scavenging, screening, repair, and programmed cell death (PCD) [10-13].

Besides this, soil and water pollution due to pesticides and herbicides has become a common concern among environmentalists. Use of pesticides and herbicides became indispensable and an integral part of modern agriculture and their use under Integrated Pest Management Programme to save the crop losses is becoming quite decisive in countries like India in the wake of second green revolution likely to be experienced in next few years. We cannot rule out the use of these chemicals (such as 2,4-D) in agricultural fields because of steady but continuous rise in population and lesser availability of agricultural fields [14]. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is used in many countries to control weeds.

Phenoxy acetic herbicides 2,4-D have auxin like activity and are used to control the growth of weeds with many crops including rice. Auxinic herbicides have been reported to be less toxic than other types of herbicides for cyanobacteria [15]. Low concentrations of 2,4-D (0.01-0.5 mM) were reported to stimulate growth and nitrogen fixation in several strains of nitrogen fixing cyanobacteria [16,17]. No stimulation, however, was reported with hormonal concentrations (10^{-1} to 10^{-4} M) of the natural auxin IAA (indole acetic acid) [18]. The toxicity range of 2,4-D varies depending on the strains and the culture conditions. Most reports indicate that cyanobacteria can readily tolerate up to 1 mM of 2,4-D. The lethal dose generally appears with herbicide concentrations ranging between 4-5 mM [15,19]. In contrast with the extensive literature regarding the interaction of 2,4-D with cyanobacteria [16, 17], little work has been done on the combine effect of herbicide 2,4-D, UVR and PAR on cyanobacteria.

In the present study, we have evaluated the possible stimulatory and/or inhibitory effect of combined exposure of UVR, PAR and 2,4-D on the roof top inhabiting desiccation tolerant cyanobacterium *Scytonema geitleri* strain HKAR-12. Survival mechanisms were investigated in terms of pigments and protein contents and antioxidant enzymes activity in response to the damage caused by combination of UVR, PAR and 2,4-D. Furthermore, information obtained with these photosynthetic organisms can be useful in understanding the mode of action of phenoxy acetic herbicides on higher plants. Cyanobacteria are a prokaryotic abundant group of primary producers and constitute the base of the trophic webs. Furthermore, they can fix atmospheric nitrogen into bioavailable forms, so that they are an important source of bioavailable nitrogen for many ecosystems [20]. Therefore, any detrimental effect on cyanobacteria may have an impairing negative effect on productivity of aquatic and terrestrial ecosystems; hence, present investigation becomes important for understanding survival strategies adapted by these organisms under combination of various stresses.

MATERIALS AND METHODS

Sampling and cultivation

Scytonema geitleri strain HKAR-12 (Accession number: KP271353), was collected aseptically from roof top of

department of botany, Banaras Hindu University (BHU), Varanasi, UP, India, was taken in the present study. Standard microbiological techniques [21] were adopted for isolation of *Scytonema geitleri* strain HKAR-12 which was grown axenically in BG-11 medium [22] without nitrogen source in a culture room at 28 ± 2 °C, and illuminated with fluorescent light of 12 ± 2 W m⁻². *Scytonema geitleri* strain HKAR-12 is an autotrophically growing heterocystous, firmly sheathed and filamentous cyanobacterium that has pseudo branching in its filaments, a feature which is characteristic of the family scytonemataceae. Branches formed generally in between heterocyst, firm sheath, trichome single in each sheath and hormogones were present.

Experimental setup

The cyanobacterial cultures ($OD_{750\text{ nm}} = 0.6 \pm 0.2$ for a path length of 1 cm) were transferred into sterile transparent Petri dishes (75 mm) and kept in an assembled closed light chamber equipped with rotary shaker for uniform exposure to PAR and PAB with 100 µg/mL 2,4-D. Two cut-off filter foils of 395 nm (Ultraphan, UV Opak Digefra, Munich, Germany) and 295 nm (Ultraphan, Digefra, Munich, Germany) were used to get the desired radiation regimes of PAR and PAB respectively. The irradiation on surface of vessel was maintained at 12 ± 2 W m⁻² for PAR, UV intensity of ~ 0.5 W m⁻² for UV-B and 1.0 W m⁻² for UV-A. During course of treatment (up to 72 h), all experimental cultures were exposed with constant temperature of 25 ± 2 °C. Subsequently, desired amounts (50 mL) of samples were withdrawn at regular intervals after 12 h of exposure and then harvested by centrifugation and subjected to further study.

Chlorophyll a (Chl a) and total carotene analysis

Pigments were analyzed taking equal amount (5mL) of sample, extracted in 90% methanol (v/v) and the absorption spectra were measured in a Hitachi U-2190 UV-Visible double beam spectrophotometer in the wavelength range of 200-800 nm using quartz cuvettes. Quantity of Chl a and total carotene was estimated using the methodology of Dere et al. [23].

Determination of proteins

Total soluble proteins were measured by the method as described by Lowry et al. [24]. Bovine serum albumin was used as standard for quantification of proteins.

Determination of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity

For assaying antioxidant enzymes, cells from UV irradiated samples and controls were harvested by centrifugation at 12,000 g for 15 min at room temperature. Cell extracts were prepared by sonicating cells in 2 mL of extraction buffer under ice-cold conditions. The extraction buffer consisted of 50 mM potassium phosphate buffer (pH 7.5), 1mM ethylene diamine tetracetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVP), 0.5% (w/v) Triton X-100 with the addition of 1 mM ascorbate in the APX assay. The homogenate was centrifuged at 10,000g for 10 min at 4 °C, and the supernatants were collected and used for assays of SOD, CAT and APX. CAT and APX activity was determined according to the methods of Aebi [25] and Nakano and Asada

[26] respectively. SOD activity was measured by monitoring the inhibition in reduction of nitro blue tetrazolium (NBT) as described previously by Fridovich [27] and Beyer and Fridovich [28].

Statistical analysis

The experiments were repeated thrice for accuracy of the results. All results were presented as mean values of three replicates. Statistical analyses were done by one way analysis of variance (ANOVA). Once a significant difference was detected post-hoc multiple comparisons were made by using the Tukey test (SPSS 16.0). The level of significance was set at 0.05 for all tests.

RESULTS AND DISCUSSION

Effects of UVR and 2,4-Don Chl *a* and total carotene content

Chl *a* and total carotene content of cyanobacterial cells varied with the duration of PAR, PAR+ 2,4-D and PAB + 2,4-D exposure as depicted in Figure (1) and Figure (2). The initial values (8.011 µg/gfw) of Chl *a* increased up to 36h (11.120 µg/gfw) of PAR exposure and thereafter declined to approximately 35% (5.21 µg/gfw) and 45.63% (4.35 µg/gfw) under PAR and PAR+2,4-D after 72h of exposure respectively. As duration of PAB and PAB + 2,4-D exposure increased, Chl *a* content of cyanobacterial cells decreased ($P < .005$). Maximum decrease was observed under PAB and PAB+ 2,4-D after 72h of treatment. Decrease upto 61.42% and 73.53% was reported in both conditions as compared to the control (8.011 µg/gfw).

Initial level of total carotene content was recorded to be 2.177 mg/gfw. However, a progressive increase in carotene content was observed after increasing duration of PAR, PAR+2,4-D, PAB and PAB+ 2,4-D exposure which was about 17.58%, 41.93%, 57.55% and 72.48% respectively after 36h of exposure. However, a subsequent decline in the level of carotene content was reported. Carotene content declined upto 72h and maximum decline was observed after 72h of PAB (63.25%) and PAB+ 2,4-D (81.6%) respectively.

Effect of UVR and 2,4-Don total protein content

The initial protein content (0.933 mg/mL) was found to be adversely affected by PAB and PAB+ 2,4-D treatment upto 72h of exposure (Figure 3). Decrease of approximately 68.0% (0.301 mg/mL) and 76.0% (0.224 mg/mL) protein content was observed ($P < .005$) after 72h of PAB and PAB+ 2,4-D exposure respectively as compared to the control (0.933 mg/mL).

Effect of UVR and 2,4-Don antioxidative enzymes activity

Figures (4-6) depicts the activities of antioxidant enzymes in *Scytonema geitleri* strain HKAR-12. The studied enzymatic antioxidants, i.e. SOD (EC 1.15.1.1), CAT (EC 1.11.1.6) and APX (1.11.1.11) showed differential responses when treated with PAR, PAR+2,4-D, PAB and PAB+2,4-D stress. Significant changes in the levels of studied antioxidative enzymes were observed in *Scytonema geitleri* strain HKAR-12. SOD activity showed about 45.71% (0.306 U mg⁻¹ of protein) increase in PAR, 65.23%

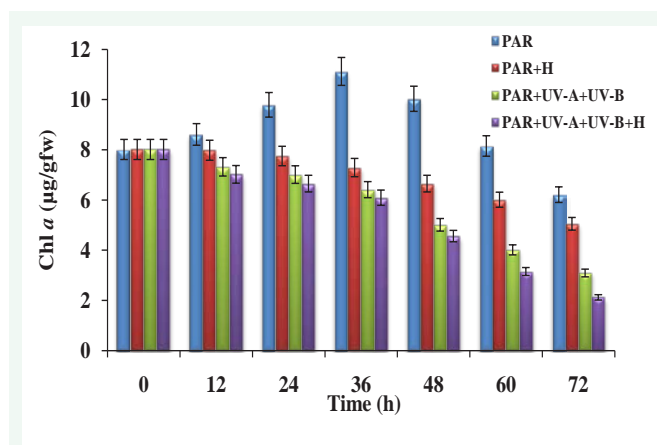


Figure 1 Effect of 2,4-D in combination with PAR and PAB on Chl *a* content of *Scytonema geitleri* strain HKAR-12 after varying duration of exposure. H represents 2,4-D. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means.

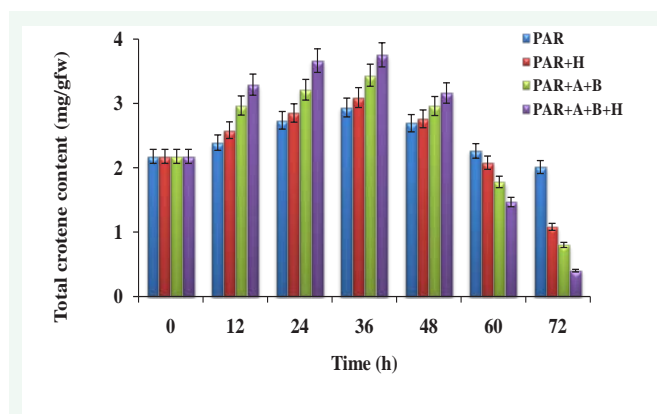


Figure 2 Effect of 2,4-D in combination with PAR and PAB on total carotene content of *Scytonema geitleri* strain HKAR-12 after varying duration of exposure. H represents 2,4-D. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means.

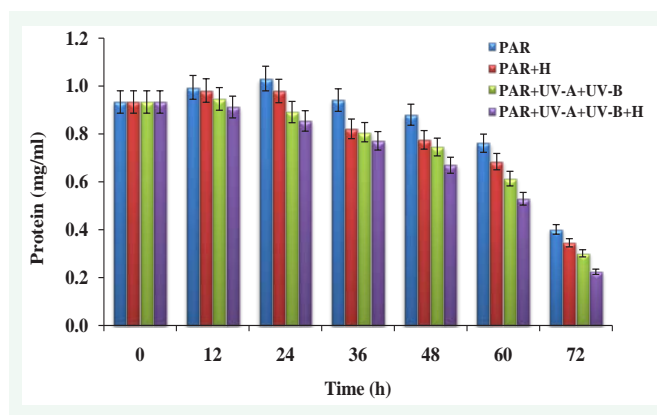


Figure 3 Effect of 2,4-D in combination with PAR and PAB on protein profile of *Scytonema geitleri* strain HKAR-12 after varying duration of exposure. H represents 2,4-D. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means.

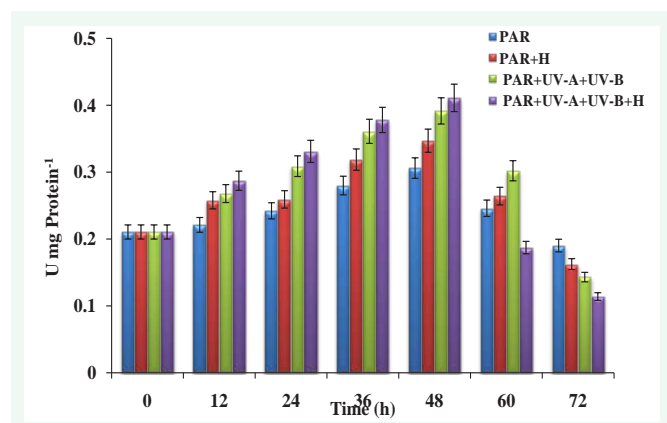


Figure 4 Effect of 2,4-D in combination with PAR and PAB on antioxidative enzyme superoxide dismutase (SOD) in *Scytonema geitleri* strain HKAR-12 after varying duration of exposure. H represents 2,4-D. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means.

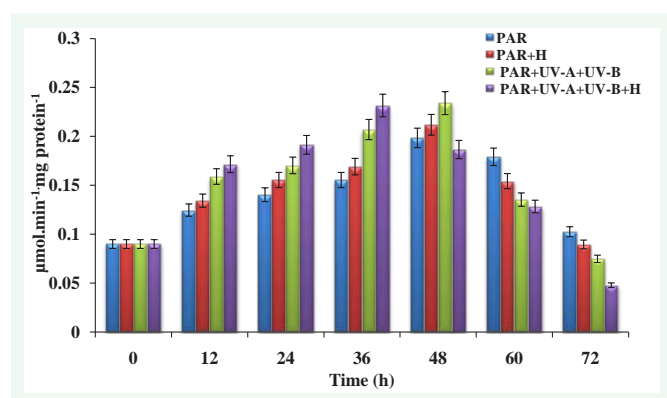


Figure 5 Effect of 2,4-D in combination with PAR and PAB on antioxidative enzyme catalase (CAT) in *Scytonema geitleri* strain HKAR-12 after varying duration of exposure. H represents 2,4-D. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means.

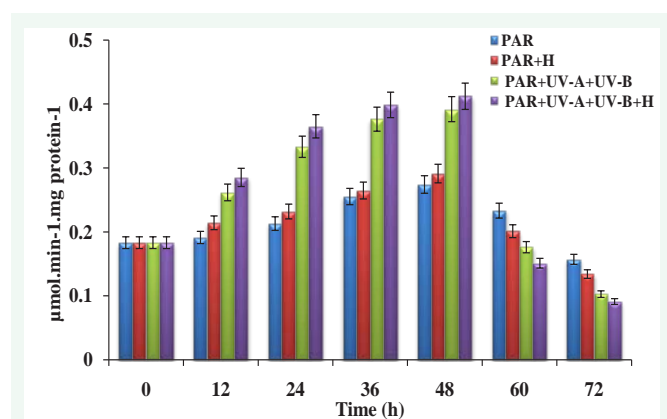


Figure 6 Effect of 2,4-D in combination with PAR and PAB on antioxidative enzyme ascorbate peroxidase (APX) in *Scytonema geitleri* strain HKAR-12 after varying duration of exposure. H represents 2,4-D. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means.

(0.347 U mg⁻¹ of protein) in PAR+2,4-D, 86.42% (0.391 U mg⁻¹ of protein) in PAB and 95.7% (0.411 U mg⁻¹ of protein) increase in PAB+2,4-D exposure in comparison to the control (0.210 U mg⁻¹ of protein) after 48 h of treatment, thereafter a decline in activity was observed ($p < 0.05$) (Figure 4).

CAT activity showed the similar trend as that of SOD. Initially an increase in the CAT activity was observed in comparison to the untreated control culture. In PAR small increase in CAT activity was observed upto 48 h of treatment thereafter it became constant (Figure 5). CAT activity increased upto 157% (0.2416 μmol.min⁻¹ mg protein⁻¹) after 36 h of PAB+2,4-D exposure and increase of about 137% (0.214 μmol.min⁻¹ mg protein⁻¹) of PAB exposure after 48 h and 120% (0.198 μmol.min⁻¹ mg protein⁻¹) in PAR after 48 h of treatment thereafter the activity began to decline as compared to the control (0.09 μmol.min⁻¹ mg protein⁻¹).

Similarly APX activity increased upto 125% (0.412 μmol.min⁻¹ mg protein⁻¹) in PAB+2,4-D treatment, 113% (0.391 μmol.min⁻¹ mg protein⁻¹) in PAB followed by 59% (0.291 μmol.min⁻¹ mg protein⁻¹) in PAR+2,4 D and 50% (0.274 μmol.min⁻¹ mg protein⁻¹) in PAR treatment after 48 h of exposure as compared to the control (0.183 μmol.min⁻¹ mg protein⁻¹) thereafter it started decreasing in all experimental setups (Figure 6).

Herbicides are environmental pollutants of high concern in terrestrial and freshwater environments due to their ubiquity resulting from their extensive use in modern agriculture and their persistence. The 2,4-D, a very common hormone weed killer, is used in paddy fields to control weeds. Its field dose is about 40 μg/mL [29]. Herbicides have detrimental effects on growth, photosynthetic pigments, protein content and oxidative stress in cyanobacterial cells [30,31]. The use of herbicides may decrease pigment content, destroy chloroplasts, thylakoids and photosystem II (PSII), and even cause DNA damage in organisms [32-34] which suggested that the damage process may be due to the ROS generation [35,36]. Loss of pigments might be another reason to protect against ROS generation and inhibition of photosynthetic electron transfer [37]. Our results showed that UVR and 2,4-D caused serious damage to Chl *a*, total carotene and protein content in *Scytonema geitleri* strain HKAR-12. However, aquatic and terrestrial organisms have developed a number of repair and tolerance mechanisms to counteract the damaging effects of UVR [13,38] along with herbicides. The synthesis of antioxidative enzymes such as SOD, CAT and APX is one such line of defense mechanisms in the test organism. Our study suggests that enzymatic defense mechanisms confer protection to the organism under UVR and 2,4-D stress. A multifold induction in the antioxidative enzymes was observed. *Scytonema geitleri* HKAR-12 was found to be more potent in the induction of SOD after 48 h of PAB along with 2,4-D exposure as compared to the control. Similar trend was observed in case of other antioxidative enzymes such as CAT and APX. However, the activity of CAT under UVR and 2,4-D stress was not significant as compared to the other studied antioxidative enzymes. Probably, relatively higher levels of antioxidant enzyme activity even after 48 h of prolonged UVR and 2,4-D exposure might contribute to the higher survival. The present study clearly suggests the role enzymatic defense mechanisms in conferring protection under UVR and herbicide stress.

The antioxidative enzymes SOD, CAT and APX showed a multifold induction upto 48h of UVR and 2,4-D exposure and were responsible for the survival even after 48h of continuous stress in *Scytonema geitleri* strain HKAR-12. A gradual decrease in their concentration was noticed after 48h of exposure suggesting that they may not be effective for a longer duration. The increasing duration of UVR and 2,4-D resulted in the decreased survival of the test organisms. Hence, lower values of all the studied parameters were recorded with increasing duration of exposure time. However, the cells maintained a basal level of the studied parameters even after prolonged UVR and 2,4-D exposure upto 48h and was able to cope up the detrimental effects of the highly energetic radiation and 2,4-D stress. Extensive large-scale use of pesticides and herbicides in agriculture for improving crop yield has caused concern among environmentalists. Herbicides used in agricultural practices are transported to water bodies through run-off, drift and leaching, which increase the risk of exposure in non-target organisms [30]. Some herbicides cause metabolic alterations in algae due to oxidative stress [35], and others function via binding to the exchangeable quinone site in the photosystem II (PSII) reaction center, thus blocking electron transfer [39].

CONCLUSION

The present work suggests that treatment of cyanobacterial cells with UVR and herbicide causes severe damage to these organisms. Extensive large-scale use of pesticides and herbicides in agriculture for improving crop yield has caused concern among environmentalists.

In the present study strongest modifying effect was found for PAB whose toxicity increased with 100 µg/mL 2,4-D exposure. UVR and herbicides have detrimental effects on growth, photosynthetic pigments, protein content and oxidative stress in cyanobacterial cells. Several studies have dealt with co-exposure of UVR with a variety of herbicides, and different patterns of interactions have been found depending on the tested cyanobacteria [14,40]. These results suggest that the combination of UV-B and exogenous herbicides have detrimental effects on cyanobacterial metabolism through either a ROS-mediated process or by affecting the electron transport chain and may cause serious damage to cyanobacteria [40, 41].

Our study shows the inhibitory effect of UVR and herbicide 2,4-D on the cyanobacterium *Scytonema geitleri* HKAR-12 and effect of one stress is aggravated by other stress and *vice-versa*. These results suggest that UVR and herbicide 2,4-D have different degree and different mechanisms of toxicity on *Scytonema geitleri* HKAR-12 through either a ROS-mediated process or by affecting the Chl *a*, carotene and total protein content. Thus, the combination of UVR and herbicides may cause serious damage to cyanobacteria and decrease the growth rate of cyanobacterial population hence more studies are needed for proper understanding of the use of herbicides and outcomes of its interactions with other abiotic stresses in cyanobacteria.

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REFERENCES

1. Stanier RY, Cohen-Bazire G. Phototrophic prokaryotes: the cyanobacteria. *Annu Rev Microbiol.* 1977; 31: 225-274.
2. Richa, Sinha RP. Sensitivity of two Nostoc species harbouring diverse habitats to ultraviolet-B radiation. *Microbiology.* 2015; 84: 398-407.
3. Vaishampayan A, Sinha RP, Hader DP, Dey T, Gupta AK, Bhan U, et al. Cyanobacterial biofertilizers in rice agriculture. *Bot Rev.* 2001; 67: 453-516.
4. Fischer WW. Biogeochemistry: Life before the rise of oxygen. *Nature.* 2008; 455: 1051-1052.
5. Häder DP, Sinha RP. Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact. *Mutat Res.* 2005; 1: 221-233.
6. Wang JG, Zhang CL. DNA damage and repair of two ecotypes of *Phragmites communis* subjected to water stress. *Acta Bot Sin.* 2001; 43: 490-494.
7. He YY, Häder DP. UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium *Anabaena* sp.: protective effects of ascorbic acid and N-acetyl-L-cystein. *J Photochem Photobiol B.* 2002; 66: 115-124.
8. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 2004; 55: 373-399.
9. Holzinger A, Lütz C. Algae and UV irradiation: effects on ultrastructure and related metabolic functions. *Micron.* 2006; 37: 190-207.
10. Quesada A, Vincent WF. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *Eur J Phycol.* 1997; 32: 335-342.
11. Gröniger A, Sinha RP, Klisch M, Häder DP. Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae--a database. *J Photochem Photobiol B.* 2000; 58: 115-122.
12. Sinha RP, Häder DP. UV-protectants in cyanobacteria. *Plant Sci.* 2008; 174: 278-289.
13. Singh SP, Häder DP, Sinha RP. Cyanobacteria and ultraviolet radiation (UVR) stress: mitigation strategies. *Ageing Res Rev.* 2010; 9: 79-90.
14. Sheeba, Singh VP, Srivastava PK, Prasad SM. Differential physiological and biochemical responses of two cyanobacteria *Nostoc muscorum* and *Phormidium foveolarum* against oxyfluorfen and UV-B radiation. *Ecotoxicol Environ Saf.* 2011; 74: 1981-1993.
15. Stratton GW. The effects of pesticides and heavy metals towards phototrophic microorganisms. *Rev Environ Toxicol.* 1987; 3: 71-147.
16. Das B, Singh PK. The effect of 2,4-dichlorophenoxyacetic acid on growth and nitrogen-fixation of blue-green alga *Anabaenopsis raciborskii*. *Arch Environ Contam Toxicol.* 1977; 5: 437-445.
17. Tiwari DN, Pandey AK, Mishra AK. Action of 2,4-dichlorophenoxy

- acetic acid and rifampicin on heterocysts differentiation in blue-green alga *Nostoclinckia*. *J Biosci*. 1981; 3: 33-39.
18. Leganes F, Sanchez-Maeso E, Fernandez-Valiente E. Effect of indoleacetic acid on growth and dinitrogen fixation in cyanobacteria. *Plant Cell Physiol*. 1987; 28: 529-533.
 19. Padhy RH. Cyanobacteria and pesticides. *Res Rev*. 1985; 95: 1-44.
 20. Sohm JA, Webb EA, Capone DG. Emerging patterns of marine nitrogen fixation. *Nat Rev Microbiol*. 2011; 9: 499-508.
 21. Sinha RP, Lebert M, Kumar A, Kumar HD, Häder DP. Disintegration of phycobilisomes in a rice field cyanobacterium *Nostoc* sp. following UV irradiation. *Biochem Mol Biol Int*. 1995; 37: 697-706.
 22. Rippka R, Deruelles J, Waterbury JB. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol*. 1979; 111: 1-61.
 23. Dere S, Gunes T, Sivaci R. Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turk J Botany*. 1998; 22: 13-17.
 24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951; 193: 265-275.
 25. Aebi H. Catalase in vitro. *Methods Enzymol*. 1984; 105: 121-126.
 26. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant cell physiol*. 1981; 22: 867-880.
 27. Fridovich I. Superoxide dismutases. *Adv Enzymol Relat Areas Mol Biol*. 1974; 41: 35-97.
 28. Beyer WF Jr, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal Biochem*. 1987; 161: 559-566.
 29. Mishra AK, Pandey AB. Toxicity of three herbicides to some nitrogen-fixing cyanobacteria. *Ecotoxicol Environ Saf*. 1989; 17: 236-246.
 30. Chen Z, Juneau P, Qiu B. Effects of three pesticides on the growth, photosynthesis and photoinhibition of the edible cyanobacterium *Ge-Xian-Mi* (*Nostoc*). *Aquat Toxicol*. 2007; 81: 256-265.
 31. Cedergreen N, Spliid NH, Streibig JC. Species-specific sensitivity of aquatic macrophytes towards two herbicides. *Ecotoxicol Environ Saf*. 2004; 58: 314-323.
 32. Nielsen LW, Dahllöf I. Direct and indirect effects of the herbicides Glyphosate, Bentazone and MCPA on eelgrass (*Zostera marina*). *Aquat Toxicol*. 2007; 82: 47-54.
 33. Mañas F, Peralta L, Raviolo J, Ovando HG, Weyers A, Ugnia L, et al. Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. *Environ Toxicol Pharmacol*. 2009; 28: 37-41.
 34. Ventrella A, Catucci L, Agostiano A. Herbicides affect fluorescence and electron transfer activity of spinach chloroplasts, thylakoid membranes and isolated Photosystem II. *Bioelectrochemistry*. 2010; 79: 43-49.
 35. Romero DM, deMolina MCR, Juarez AB. Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of *Chlorella kessleri*. *Ecotoxicol Environ Saf*. 2011; 74: 741-747.
 36. Cedergreen N, Olesen CF. Can glyphosate stimulate photosynthesis? *PesticBiochem Phys*. 2010; 96: 140-148.
 37. Bhattacharyya S, Nayak B, Choudhury NK. Response of diazotrophic cyanobacterium *Nostoc carneum* under pesticide and UV-B stress. *Chemosphere*. 2011; 84: 131-135.
 38. Quesada A, Vincent WF. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *Eur J Phycol*. 1997; 32: 335-342.
 39. Rutherford AW, Krieger-Liszka A. Herbicide-induced oxidative stress in photosystem II. *Trends Biochem Sci*. 2011; 26: 648-653.
 40. Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y. The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. *Ecotoxicol Environ Saf*. 2012; 80: 224-230.
 41. Rodea-Palomares I, Makowski M, Gonzalo S, González-Pleiter M, Leganés F, Fernández-Piñas F. Effect of PFOA/PFOS pre-exposure on the toxicity of the herbicides 2, 4-D, Atrazine, Diuron and Paraquat to a model aquatic photosynthetic microorganism. *Chemosphere*. 2015; 139: 65-72.

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