Chapter-3

Experimental Findings

(A) Growth and related physiological and biochemical responses

3.1 Growth behaviour and photosynthetic pigments

3.1.1 Growth behaviour

The growth behavior of the tested cyanobacteria viz. *Nostoc muscorum* and *Phormidium foveolarum* was monitored by observing changes in absorbance at 750 nm, relative growth rate, dry weight accumulation and protein content after 24 and 72 h of experiments. The cyanobacteria were treated with 1 and 2 µg ml\(^{-1}\) of chlorpyrifos (denoted as CP\(_L\) and CP\(_H\), respectively), and just after chlorpyrifos treatments cells were exposed with low (UV-B\(_L\); 0.1 µmol m\(^{-2}\) s\(^{-1}\)) and high (UV-B\(_H\); 0.1 µmol m\(^{-2}\) s\(^{-1}\)) fluence rates of UV-B radiation for 4 h each day at the middle of the photoperiod. After 24 and 72 h of experiments, parameters related to growth in both the cyanobacteria were determined. In the present study, chlorpyrifos concentrations *i.e.* CP\(_L\) and CP\(_H\) and low and high fluence rates of UV-B radiation were selected on the basis of series of screening experiments (Figs. 1-4).

Absorbance (\(\lambda_{750}\)) provides direct measurement of growth in aqueous culture. Data revealed that chlorpyrifos at CP\(_L\) and CP\(_H\) doses, decreased growth by 18 and 44% in *N. muscorum* while under similar condition it declined by 13 and 28% in *P. foveolarum*, respectively after 24 h of experiment when compared with the control (Fig. 5). After 72 h, CP\(_H\) dose remained inhibitory as it caused 32 and 20% reduction in *N. muscorum* and *P. foveolarum*, respectively, however during this period CP\(_L\) dose became stimulatory and gained 8 and 4% more growth in *N. muscorum* and *P. foveolarum*, respectively over their respective controls. UV-B radiation at high fluence rate (UV-B\(_H\)) also produced severe damage to growth as the absorbance (\(\lambda_{750}\)) decreased by 23% in *N. muscorum* and
by 13% in *P. foveolarum* after 24 h of experiment. These results reveal that *N. muscorum* was more sensitive to chlorpyrifos and UV-B in comparison to *P. foveolarum*. In contrast, UV-B radiation at low fluence rate (UV-B\(_L\)) alone did not cause any notable change in growth of both the organisms, but when UV-B\(_L\) was given together with chlorpyrifos, CP\(_L\) dose-induced growth inhibition was reduced by about 6% in *N. muscorum* as well as in *P. foveolarum* after 24 h of experiments. Similarly, CP\(_H\) induced inhibition was reduced by 10 and 7% by UV-B\(_L\) dose in *N. muscorum* and *P. foveolarum*, respectively after 24 h of experiment.

Relative growth rate (RGR) gives the instantaneous picture of the growth behavior of the organism. Results pertaining to RGR have been shown in Fig. 6. Relative growth rate was found to be 0.683±0.019 d\(^{-1}\) in control cells of *N. muscorum* and reduced by about 29% and 86% after CP\(_L\) and CP\(_H\) dose of chlorpyrifos application, respectively in 24 h while after 72 h, 5% more growth was noticed in the case of CP\(_L\) dose alone and the inhibition persisted only 26% in the case of CP\(_H\) dose. On the contrary, in the case of *P. foveolarum*, the RGR declined up to 17 and 40% by CP\(_L\) and CP\(_H\) dose, respectively in 24 h. After 72 h, CP\(_L\) dose treated cells gained growth marginally by 2% and reduction in growth was limited to 14% in the case of CP\(_H\) dose treatment. Low dose of UV-B (UV-B\(_L\)) could cause a meagre 3 and 2% inhibition in *N. muscorum* and *P. foveolarum* cells, respectively whereas its higher dose (UV-B\(_H\)) caused 39 and 17% decline in *N. muscorum* and *P. foveolarum* cells, respectively. By the time, after 72 h of UV-B\(_L\) exposed cells appeared to recover upto control level. The Fig. 6 reveals that CP\(_L\) dose was initially toxic but becomes stimulatory after 72 h of estimation. CP\(_H\) dose remained inhibitory even after 72 h of experiment. Similarly, UV-B\(_L\) was also seemed to be growth inhibitory in the beginning of the experiment but the exposed cells recovered successfully later, while UV-B\(_H\) remained inhibitory even after 72 h of measurement. When UV-B\(_L\) was combined with any of the CP doses, it caused enhancements by various degrees in initial RGR of the cyanobacterial samples. On the other hand, when UV-B\(_H\) was exposed to CP treated cells, either enhancements/recovery were significantly diminished or inhibitory effects become worsen. The interesting side of the finding is that the RGR
either get enhanced or recovered from damaging effects of the insecticide following UV-B<sub>L</sub> exposure.

Impact of the individual as well as twin stresses on dry weight (biomass accumulation) of both the cyanobacteria has been portrayed in Fig. 7. The data pertaining to the figure clear that CP<sub>L</sub> and CP<sub>H</sub> exposure decreased dry weight by 18 and 44% in *N. muscorum* and by 13 and 28%, respectively in *P. foveolarum* after 24 h of experiment. After 72 h of experiment, an increase of 8 and 4% in dry weight over the values of untreated control cells was found in *N. muscorum* and *P. foveolarum*, respectively after CP<sub>L</sub> dose treatment. On the other hand, CP<sub>H</sub> dose remained inhibitory, but the inhibitions were alleviated significantly showing only 32 and 20% reduction in *N. muscorum* and *P. foveolarum*, respectively. Similarly the other stress *i.e.* UV-B<sub>H</sub> decreased dry mass by 23% in *N. muscorum* and by 13% in *P. foveolarum* after 24 h of experiment. Combined treatment of the insecticide and UV-B<sub>H</sub> further exacerbated decrease in biomass accumulation. Maximum decrease in dry weight was observed in *N. muscorum* under the treatment of CP<sub>H</sub>+UV-B<sub>H</sub> after 24 h of experiment; however, after 72 h of experiment decrease in dry weight was lowered appreciably. The UV-B<sub>L</sub> dose alone did not bring about any notable change in dry weight accumulation in both the organisms after 24 h but whenever this dose was given in combination with either dose of the chlorpyrifos, either it caused stimulation in biomass accumulation or it significantly reduced the chlorpyrifos induced inhibitory effects.

Results pertaining to protein have been depicted in Fig. 8. Data pertaining to protein revealed that CP<sub>L</sub> caused stimulations in the protein content of *N. muscorum* cells only while CP<sub>H</sub> decreased it substantially in both the organisms, after 24 h of experiment. However, after 72 h of experiment, when only CP<sub>L</sub> dose was given, a marked increase in protein content was found in both the tested cyanobacteria. On the other hand, CP<sub>H</sub> dose remained inhibitory. UV-B<sub>H</sub> dose alone decreased protein content by 36% in *N. muscorum* and by 23% in *P. foveolarum* after 24 h of experiment. Combined treatment of UV-B<sub>H</sub> with CP<sub>H</sub> dose of the insecticide further exacerbated decrease in protein content during experimental period. Maximum decrease in protein content was observed in *N.
*muscorum* under CP*H*+UV-B*H* treatment after 24 h of experiment; however, after 72 h of experiment an appreciable recovery took place in all cyanobacterial samples in comparison to their corresponding 24 h ally. The UV-B*L* dose alone was able to bring about significant increment in protein content after 72 h of experiment in *N. muscorum* as well as *P. foveolarum*. When this UV-B*L* dose was given in combination with either of the chlorpyrifos doses, after 72 h of experiments, either it caused stimulation (as in case of CP*L* dose) or it significantly alleviated the chlorpyrifos induced inhibitory effects on protein (as in case of CP*H* dose).

### 3.1.2 Photosynthetic pigments

Chlorophyll *a* and phycocyanin are the prominent light harvesting photosynthetic pigments of cyanobacteria. Carotenoids act as accessory pigments and are protective in nature. Among the photosynthetic pigments, carotenoids are known to protect the photoautotrophs from photodynamic damage, and therefore they act as defense system under stress conditions.

Results pertaining to chlorophyll *a*, carotenoids and phycocyanin of *N. muscorum* and *P. foveolarum* have been arranged in Table 1 and 2, respectively. The control cells of *N. muscorum* showed 6.40±0.18 µg Chl *a* mg⁻¹ DW when measured after 24 h, and reached 11.00±0.32 µg Chl *a* mg⁻¹ DW, after 72 h of experiment. On the other hand, control cells of *P. foveolarum* contained 6.70±0.19 µg Chl *a* mg⁻¹ DW and after 72 h the amount was 11.80±0.34 µg Chl *a* mg⁻¹ DW. CP*L* dose caused about 18% inhibition while CP*H* dose caused a substantial decrease of 39% in *N. muscorum*, after 24 h of experiment. Later on, after 72 h of Chl *a* estimation, *N. muscorum* treated with CP*L* dose exhibited a considerable increment of 10%, contrary to *P. foveolarum* which could show a gain of only 6% in Chl *a* suggesting that *P. foveolarum* resists the changes. Again, after CP*H* exposure to the tested cyanobacteria, reduction in Chl *a* in *N. muscorum* were recorded more than those recorded in *P. foveolarum*. Any marked alteration in Chl *a* content could not be noticed in UV-B*L* treated test organisms whether measured after 24 h or 72 h but when it combined with any of the CP doses, there was an increase in Chl *a* content in CP*L*
treated cells or significantly reduced the CP_H induced inhibitions in Chl a content. Whenever UV-B_H combines with any of the CP doses, it reduced the Chl a content further.

Carotenoids and phycocyanin contents also display the similar pattern of change (Tables 1 and 2). After exposure with CP_L dose, an initial reduction in both the pigments was seen followed by stimulation. Individual effect of UV-B_L was found to be stimulatory on carotenoids but insignificantly inhibitory on phycocyanin from very setting-off the experiment. Never the less, UV-B_L caused increase in these pigments when combined with CP_L dose and reduced the inhibitory effects of CP_H dose appreciably. Again UV-B_H caused severe damage in these pigments as N. muscorum scored 2.15±0.06 µg Car mg^{-1} DW while P. foveolarum had 2.50±0.07 µg Car mg^{-1} DW showing a decrease of 14% in N. muscorum and 11% in P. foveolarum in carotenoids content. Phycocyanin contents were recorded as 28.41±0.82 µg mg^{-1} DW in N. muscorum and 33.39±0.96 µg mg^{-1} DW in P. foveolarum resulting 27% decrease in N. muscorum and 24% decrease in P. foveolarum in comparison to their respective controls. Overall data pertinent to pigments establish P. foveolarum to be more resistant than N. muscorum.

### 3.2 Photosynthesis and respiration

(I) Photosynthesis

Life on earth ultimately depends on energy derived from the sun. Photosynthesis is the only physiological process which can harvest this energy. Photosynthesis is key to growth and biomass accumulation in photoautotrophs. Besides photosynthetic pigments, the primary photosynthetic reactions are also considered to be the direct target of pesticides and UV-B. Considering these facts, in the present study, impact of low and high fluence rates of UV-B radiation was analyzed on photosynthetic oxygen yield, photosynthetic electron transport activities and spectrophotometric assay of PS II activity in terms of DCPIP photoreduction by measuring changes in absorption at 600 nm in the presence of various exogenous electron donors *i.e.* hydroxylamine (NH$_2$OH), diphenyl carbazide (DPC) and manganese chloride (MnCl$_2$).
3.2.1 Photosynthetic oxygen yield

Results pertaining to photosynthetic oxygen yield have been portrayed in Fig. 9. The level of photosynthetic oxygen yields were found to be 72.00±2.08 µmol O$_2$-evolved (g DW min)$^{-1}$ for *N. muscorum* and 87.00±2.51 µmol O$_2$-evolved (g DW min)$^{-1}$ in case of *P. foveolarum* after 24 h of experiment. CP$_L$ was able to decline photosynthetic oxygen yield by 18% in *N. muscorum* while it could reduce the O$_2$ yield by 11% in *P. foveolarum*. Further, CP$_L$ dose appeared to be stimulatory as after 72 h of estimation of photosynthetic oxygen yield, 5 and 9% more rates were recorded in *N. muscorum* and *P. foveolarum*, respectively in comparison to their respective control cells. CP$_H$ remained inhibitory from very beginning and caused 42% inhibition in *N. muscorum* and 23% inhibition in *P. foveolarum*, after 24 h of monitoring. Similarly, UV-B$_H$ suppressed photosynthetic oxygen evolution by 21% in *N. muscorum* and by 17% in *P. foveolarum*. Combined treatment of CP$_H$ and UV-B$_H$ further declined O$_2$-yield in both the organisms compared to their individual treatments. However, a marked recovery was seen in CP$_H$ and/or UV-B$_H$ induced inhibitions after 72 h of experiment when compared with 24 h of measurements. UV-B$_L$ did not show suppression in O$_2$-evolution in case of its individual exposure while after combining with CP$_L$, it significantly alleviated CP$_L$ induced reduction in O$_2$-evolution.

3.2.2 Photosynthetic electron transport activities (PS II, PS I and whole photosynthetic chain reaction)

Apart from indirect effects of reduction in light harvesting complex, the decline in the photosynthetic activity are more likely to be the results of disturbances in the primary photosynthetic electron transport activities. Therefore, for the characterization of site of inhibition in photosynthesis under CP$_H$ and UV-B$_H$ stresses, the activity of various components of photosynthetic electron transport chain *i.e.* PS II, PS I and whole photosynthetic chain reaction were determined in the spheroplasts of tested cyanobacteria. Immediately after spheroplasts preparation, the PS I and whole photosynthetic chain reaction (in terms of O$_2$-consumption) and PS II (in terms of O$_2$-evolution) were determined.
Results pertaining to PS II, PS I and whole chain electron transport activity have been shown in Tables 3 and 4. Results revealed that CP at both doses (1 and 2 µg ml⁻¹) significantly decreased PS II, PS I and whole photosynthetic chain reaction in both the organisms. Chlorpyrifos at 1 and 2 µg ml⁻¹ concentrations decreased PS II activity by 20 and 43%, PS I activity by 14 and 37% and whole photosynthetic chain reaction by 34 and 47% in N. muscorum, respectively after 24 h of experiment. This suggests that in N. muscorum whole chain photosynthetic activity was the most vulnerable to CP stress followed by PS II and PS I activities. Under similar treatments, in P. foveolarum PS II activity declined by 12 and 30%, PS I by 9 and 24% and whole chain photosynthetic reaction was declined by 28 and 39%, respectively after 24 h of experiment. Though, in P. foveolarum also, whole chain photosynthetic activity was the most prone to CP stress, but the findings pertaining to photosynthetic electron transport activities establishes that these activities of P. foveolarum were a bit resistant in comparison to those of N. muscorum. After 72 h of experiment, a positive impact of CP₄ dose on photosynthetic electron transport activities was seen in both the organisms as they registered a significant increment of 7-14% in these activities. In addition to this, the tested cyanobacteria showed considerable recovery in the extent of inhibition in PS II, PS I and whole chain photosynthetic reactions caused by CP₄ dose after 72 h of experiments. Exposure of UV-B₄ also produced negative effect on these electron transport activities. In N. muscorum, UV-B₄ decreased PS II, PS I and whole chain photosynthetic reaction by 29, 21 and 32% and in P. foveolarum by 23, 17 and 26%, respectively after 24 h of experiment, again suggesting the tolerant nature of PS I activity. However, after 72 h of experiment, tested cyanobacteria exhibited appreciable decrease in inhibitions in PS II, PS I and whole chain photosynthetic reactions caused by UV-B₄. Further, P. foveolarum was found to be comparatively resistant for UV-B₄ stress. In addition to this, on combining of CP₄ and UV-B₄, the suppression in electron transport activities further increased compared to their individual treatments. CP₄+UV-B₄ declined the activity of PS II, PS I and whole chain photosynthetic reaction by 54, 43 and 67% in N. muscorum and by 36, 33 and 54% in P. foveolarum, respectively after 24 h of experiment. On the
contrary, UV-B_L alone insignificantly diminished all the three electron transport activities after 24 h and showed a tendency for improvement after 72 h of experiment. But whenever UV-B_L was given in addition to differential CP treatments, an appreciable amelioration of CP induced inhibitory effects was seen, after 24 as well as 72 h of experiments. In conclusion, whole chain activity appeared to be more vulnerable to CP_H and UV-B_H stresses, followed by PS II and PS I. Results also ascertain that these activities were more affected in *N. muscorum* than *P. foveolarum*.

### 3.2.3 Restoration in PS II activity under exogenous electron donors

To understand the possible site of action of CP_H and UV-B on PS II, PS II mediated electron transport activity in terms of DCPIP photoreduction (2, 6-dichlorophenol indophenol) was estimated in the presence of artificial electron donors *i.e.* DPC, NH_2OH, and MnCl_2 (Tables 5 and 6). These artificial electron donors are uncharged, redox sensitive compounds, so can easily transported across the membranes and donate electrons to different specific sites on the water oxidation side of PS II. They are extremely useful in measuring the electron transport activity of various segments of the photosynthetic electron transport pathway in isolated spheroplasts. Electron donors’ site specificity depends on their redox potential and on accessibility to the electron transport chain. Spheroplasts were isolated from differential CP and UV-B alone and in combination treated cells after 24 and 72 h of experiments. Addition of saturating concentrations of DPC, NH_2OH and MnCl_2 in spheroplasts restored the PS II activity upto different extent in both the organisms. Among different artificial electron donors, DPC appeared to be more effective in restoration of PS II activity.

### (II) Respiration

Being prokaryote, cyanobacteria do not possess double membrane bound cell organelles. In comparison to photosynthesis, cyanobacterial respiration is quite low. Another interesting feature is – in cyanobacteria, Krebs cycle is mainly involved in anabolic reactions because of absence of certain enzymes (2-oxoglutarate dehydrogenase)
of Krebs cycle and thus, it is interrupted. Besides this, certain components of photosynthetic electron transport chain also participate in respiratory electron transport. The results pertaining to respiration (in dark) in terms of \( \mu \text{mol O}_2 \text{-consumed (g DW min)}^{-1} \) in tested cyanobacteria have been depicted in Fig. 10. The figure shows that the rate of respiration in both the cyanobacteria got accelerated when treated with either doses of CP after 24 h of experiment. \textit{N. muscorum} exhibited decrease after 72 h of experiments however, \textit{P. foveolarum} experienced further increase in respiration. The respiration rate in control cells of \textit{N. muscorum} and \textit{P. foveolarum} was 22.00±0.64 and 25.00±0.72 \( \mu \text{mol O}_2 \text{-consumed (g DW min)}^{-1} \), respectively after 24 h of experiment. CP\textsubscript{L} and CP\textsubscript{H} increased respiration rate by 11 and 40\% in \textit{N. muscorum} and by 7 and 19\%, respectively in \textit{P. foveolarum} after 24 h of experiment. UV-B\textsubscript{H} dose alone also enhanced respiration rate as it was increased by 17\% in \textit{N. muscorum} and by 13\% in \textit{P. foveolarum} after 24 h of experiment. On combining CP\textsubscript{H} with UV-B\textsubscript{H}, the respiration rate was further increased compared to their individual treatments. Respiration rate was further increased by about 19\% when either of the CP doses treated \textit{N. muscorum} cells were simultaneously exposed with UV-B\textsubscript{H}. However, in \textit{P. foveolarum} cells, this additional inhibition was restricted up to meager 2\% in case of CP\textsubscript{L} dose but again reached up to substantive 19\% in case of CP\textsubscript{H} after 24 h of experiments. UV-B\textsubscript{L} dose alone did not significantly influence the respiration rate in both the cyanobacteria compared to their control values. But, the interesting face of the finding was that whenever UV-B\textsubscript{L} dose was given in addition to the CP\textsubscript{L} dose, it significantly enhanced the respiration rate in both the tested cyanobacteria, while on the other hand CP\textsubscript{H} dose treated cells registered a decreasing trend after additional UV-B\textsubscript{L} treatment in \textit{N. muscorum} cells, after 24 h of experiments.

### 3.3 Nutrient uptake

The inorganic ions in cells serve nutritional, osmotic, signaling and storage function. Therefore, a decrease in nutrient availability may adversely affect physiological and biochemical processes of plants. Nitrogen and phosphorus are important macronutrients controlling growth and development of cyanobacteria. They are the...
components of key molecules such as nucleic acids, lipids, proteins and ATP. Although cyanobacteria can fix molecular nitrogen, however, they also frequently use nitrate and nitrite as a source of nitrogen in natural as well as culture conditions. Chlorpyrifos and UV-B radiation may also affect cyanobacterial growth by altering uptake of mineral elements. Therefore, in the present study, impact of low and high doses of UV-B on nitrate, nitrite and phosphate uptake in tested cyanobacteria was examined under low and high doses of chlorpyrifos. For NO$_3^-$ and NO$_2^-$ uptake, in N. muscorum was also grown in BG 11 medium supplemented with NaNO$_3$.

3.3.1 Nitrate (NO$_3^-$) uptake

For NO$_3^-$ uptake rate assay, treated and untreated cyanobacterial cells were harvested after 24 and 72 h of experiments, washed thoroughly with 1% EDTA solution. Finally, cells were resuspended in 100 µM KNO$_3$ containing nutrient medium under the light intensity of 75 µmol photon m$^{-2}$ s$^{-1}$ for 4 h. The results pertaining to NO$_3^-$ uptake rate have been depicted in Fig. 11. Low dose of chlorpyrifos caused 17% decrease in N. muscorum cells while only 9% inhibition in NO$_3^-$ uptake was observed in case of P. foveolarum, after 24 h of measurements, but after 72 h of experiment, uptake rate was found to be 13 and 8% greater than their respective controls in tested organisms proving that CP$_L$ has differential stimulatory impacts on NO$_3^-$ uptake of both the organisms. CP$_H$ remained inhibitory for 24 as well as 72 h of experiments, but significant improvement was noticed during this period. Again, individual effect of UV-B$_H$ was inhibitory on this parameter while UV-B$_L$ could not cause any significant alteration. But, whenever UV-B$_L$ combined with any of the CP doses, it either tended to reduce the inhibitory impacts or provoked the stimulations. Nitrate uptake of P. foveolarum was less affected in comparison to that of N. muscorum against high doses of both the factors, UV-B$_H$ and CP$_H$.

3.3.2 Nitrite (NO$_2^-$) uptake

For NO$_2^-$ uptake rate assessment, treated and untreated cyanobacterial cells were harvested after 24 and 72 h of experiments, washed thoroughly with 1% EDTA solution. Finally cells were resuspended in 100 µM KNO$_2$ containing nutrient medium under 75
µmol photon m\(^{-2}\) s\(^{-1}\) illuminated for 4 h. Results related to NO\(_2^-\) uptake rate have been depicted in Fig. 12. Control cells of *N. muscorum* showed 44.00±1.27 µmol NO\(_2^-\) uptake (g DW h\(^{-1}\)) after 24 h of experiment and 65.00±1.88 µmol NO\(_2^-\) uptake (g DW h\(^{-1}\)) after 72 of experiment, while those of *P. foveolarum* were found to be 55.00±1.59 µmol NO\(_2^-\) uptake (g DW h\(^{-1}\)) and 83.00±2.40 µmol NO\(_2^-\) uptake (g DW h\(^{-1}\)). A considerable inhibition of 12 and 35% was noticed following the exposures of CP\(_L\) and CP\(_H\) in case of *N. muscorum* cells and 9 and 19% in *P. foveolarum*, these inhibitions were improved by 5 and 7% in *N. muscorum* and by 5 and 6% in *P. foveolarum* by simultaneous exposure of UV-B\(_L\), while UV-B\(_L\) alone could not succeed in altering NO\(_2^-\) uptake rate in both the test cyanobacteria. CP\(_H\), whether alone or in combination with either doses of UV-B, always showed detrimental effects on NO\(_2^-\) uptake rate of both the organisms after 24 h of experiments but after 72 h, the uptake rates were substantially improved in *N. muscorum* as well as in *P. foveolarum*.

### 3.3.3 Phosphate (PO\(_4^{3-}\)) uptake

For PO\(_4^{3-}\) uptake, treated and untreated (control) cells were harvested after 24 and 72 h of experiments, washed thoroughly with 1% EDTA solution. Thereafter, cells were resuspended in PO\(_4^{3-}\) (80 µM KH\(_2\)PO\(_4\)) containing medium under 75 µmol photon m\(^{-2}\) s\(^{-1}\) of PAR for 4 h. Results related to PO\(_4^{3-}\) uptake rate in both the organisms treated with low and high doses of CP and UV-B have been depicted in Fig. 13. CP\(_L\) substantially decreased PO\(_4^{3-}\) uptake rate by 46% in *N. muscorum* and by 31% in *P. foveolarum*. CP\(_H\) dose inhibited the rate more vigorously by 57 and 41% in *N. muscorum* and *P. foveolarum*, respectively. Treatment of UV-B\(_L\) meagerly increased the PO\(_4^{3-}\) uptake in *N. muscorum* as well as in *P. foveolarum* and on combining with any of the CP doses, substantially improved the CP induced inhibitions in PO\(_4^{3-}\) uptake rates.

### 3.4 Enzyme activity related with nitrogen metabolism

Nitrogen metabolism is one of the important biochemical processes of plants including cyanobacteria. Although cyanobacteria can biologically fix atmospheric nitrogen into NH\(_4^+\), however, NO\(_3^-\) is the available form of nitrogen which is most
widely used by cyanobacteria. Besides NO₃⁻, nitrite and urea, some cyanobacteria can also assimilate arginine or glutamine. For the assimilation of nitrate, an organism needs to have nitrate reductase (NR) and nitrite reductase (NiR). NR and NiR are required for the subsequent two-step reduction of nitrate into NH₄⁺. For the measurements of NR and NiR activities in heterocystous cyanobacterium N. muscorum, it was grown in BG-11 medium containing 20 mM KNO₃ or 0.2 mM NaNO₂ at beginning of experiment to induce NR and NiR enzymes, respectively. Ammonium is a form of nitrogen incorporated into carbon skeletons by photosynthetic organisms in a process known as ammonium assimilation. Ammonium is the most reduced inorganic form of nitrogen available for assimilation. Ammonium assimilation constitutes a central metabolic pathway in cyanobacteria which is linked to photosynthesis through the requirement of ATP and reducing power. Ammonium is incorporated into carbon skeletons by sequential action of two enzymes: glutamine synthetase (GS) and glutamate synthase (also called as glutamine 2-oxoglutarate aminotransferase, GOGAT), commonly known as the GS-GOGAT pathway. 2-Oxoglutarate synthesized by isocitrate dehydrogenase (IDH) is the carbon skeleton required for ammonium incorporation. Besides GS-GOGAT pathway, ammonium may also be incorporated into glutamate by aminating activity of glutamate dehydrogenase (GDH) under certain conditions. This alternative pathway of ammonium incorporation is also present in cyanobacteria. Thus, due to importance of nitrogen metabolism in growth and development of cyanobacteria, the impact of low and high fluence rate of UV-B on the activity of enzymes related to nitrogen metabolism was investigated under stimulatory and inhibitory doses of CP.

3.4.1 Nitrate reductase activity

The NR activity was studied by the estimation of NO₂⁻ formation. An in vivo assay of NR activity was performed with dithionite-reduced methyl viologen as reductant in cells made permeable by including mixed alkyltrimethylammonium bromide (MTA) in the reaction mixture after 24 and 72 h of experiments. Results pertaining to nitrate reductase activity have been shown in Fig. 14. Likewise NO₃⁻ uptake, nitrate reductase (NR) activity declined after CPₜ and CPₜₜ application when assayed after 24 h.
of treatments. CP$_H$ remained inhibitory while CP$_L$ became stimulatory after 72 h of treatments. Being stimulatory singly, UV-B$_L$ when combined with any of the insecticide doses alleviated the inhibitory effects of the insecticide in both the organisms. UV-B$_H$ was much inhibitory for the NR activity in both the organisms and further aggravated the inhibitory effects when combined with CP$_H$ dose and also checked CP$_L$ induced stimulation in NR activity.

3.4.2 Nitrite reductase activity

The NiR activity was measured as depletion of NO$_2^-$ ions from the medium. An *in vivo* assay of NiR was performed with dithionite-reduced methyl viologen as reductant in cells made permeable by mixed alkytrimethylammonium bromide (MTA) in the reaction mixture. Results related to NiR activity in both the cyanobacteria exposed to low and high fluence rates of UV-B under low and high doses of chlorpyrifos after 24 and 72 h of experiments have been shown in Fig. 15. Figure reveals that NiR activity was lowered by 20 and 46% in *N. muscorum* and by 16 and 43% in *P. foveolarum* after CP$_L$ and CP$_H$ dose application, respectively after 24 h of experiment. Under similar conditions, after 72 h of experiment, inhibitory effect of CP$_L$ was eased and 6 and 5% stimulations were observed in *N. muscorum* and *P. foveolarum*, respectively. Further, inhibition in NiR activity caused by CP$_H$ treatment after 72 h was found to be lower when compared with the values of 24 h of experiment. High fluence rate of UV-B also significantly suppressed NiR activity as it declined the activities by 26% in *N. muscorum* and by 21% in *P. foveolarum* after 24 h of experiment. However, after 72 h of experiment, considerable restoration in NiR activity was noticed. On combining of CP$_L$ or CP$_H$ with UV-B$_H$, greater decline in NiR activity was recorded when compared to their individual treatments in case of 24 h experiment, and prevented CP$_L$ dose induced stimulatory effects after 72 h of experiments. Maximum decline in NiR activity was registered in *N. muscorum* under CP$_H$+UV-B$_H$ treatment after 24 h as it scored a decline of 49% over the value of control. In contrast to UV-B$_H$ dose, UV-B$_L$ dose together with both doses of CP produced alleviation in declined NiR activity. Control cells of *N. muscorum* possessed 17.00±0.49 U mg$^{-1}$ protein NiR activity while those of *P.
foveolarum had 25.00±0.72 U mg⁻¹ protein NiR activity, when measured after 24 h of experiment and had gone upto 31.00±0.89 U mg⁻¹ protein in *N. muscorum* and 44.00±1.27 U mg⁻¹ protein in *P. foveolarum*.

3.4.3 Glutamine synthetase activity

Glutamine synthetase is the first enzyme of ammonium assimilation. The GS catalyzes the formation of glutamine by combining ammonium to glutamate. The activity of GS was estimated in *N. muscorum* and *P. foveolarum* after 24 and 72 h of experiments and results have been depicted in Fig. 16. The inherent level of GS activity was 1772.30±51.16 U mg⁻¹ protein in *N. muscorum* and 2441.80±70.49 U mg⁻¹ protein in *P. foveolarum*, assayed after 24 h and reached 2166.00±62.53 and 3032.5±87.54 U mg⁻¹ protein, respectively after 72 h of assay. GS activity remained 80-83% of their respective controls in both the tested organisms after CP₄ treatment in 24 h of experiment, but these samples exhibited 5-9% increment over the values of control when monitored after 72 h. CP₄ dose caused about 47% and 30% inhibition in *N. muscorum* and *P. foveolarum*, respectively after 24 h of experiment and after 72 h of experiment the activity improved by 15 and 10%, respectively. UV-B₄, although being insignificantly stimulatory, caused much improvement in CP doses induced inhibitions. UV-B₄ remained inhibitory for GS activity and further suppressed the CP induced inhibitions in GS activity.

3.4.4 Glutamate synthase activity

Glutamate synthase also called as glutamine 2-oxoglutarate aminotransferase (GOGAT) is the second important enzyme of ammonium assimilation pathway, catalyzes formation of two molecules of glutamate from glutamine and 2-oxoglutarate using NADH or ferredoxin as reducing power. The NADH-GOGAT activity was measured in the tested cyanobacteria after 24 and 72 h of experiments and the results have been depicted in Fig. 17. Level of GOGAT activity was 22.50±0.65 U mg⁻¹ protein in *N. muscorum* and 42.30±1.22 U mg⁻¹ protein in *P. foveolarum*, when estimated after 24 h, and after 72 h of experiment, the activity reached 36.30±1.05 U mg⁻¹ protein and 70.50±2.04 U mg⁻¹ protein, respectively. CP₄ inhibited the GOGAT activity initially, but
after 72 h promoted the activity by 11% in *N. muscorum* and by 4% in *P. foveolarum*. Results pointed out that although CP<sub>L</sub> dose caused more inhibition in *N. muscorum* cells however after 72 h the stimulation was also higher in comparison to *P. foveolarum*. UV-B<sub>L</sub> was again proved to be stimulatory either exposed singly or together with any of the CP doses. When UV-B<sub>L</sub> was given along with CP<sub>L</sub> dose, it prevented the inhibition in GOGAT activity by 9% in *N. muscorum* and by 8% in *P. foveolarum*, when measured after 24 h of experiment. UV-B<sub>H</sub> individually inhibited the GOGAT activity substantially and further aggravated the CP induced inhibitory effects. The maximum inhibition took place in 24 h following CP<sub>H</sub>+UV-B<sub>H</sub> treatment in *N. muscorum* cells.

### 3.4.5 Glutamate dehydrogenase activity

Although GDH is regarded as an enzyme of another route of ammonium assimilation as it has recently been shown that when primary route of ammonium assimilation *i.e.* GS-GOGAT pathway is obstructed partially/completely under stress conditions, the aminating activity of GDH plays an important role in relieving the pressure of accumulating ammonium ions. Ammonium ions become toxic when tend to accumulate in cell. Under such condition, aminating activity of GDH produces glutamate to fulfill requirement of the cells as GDH has high Km value for ammonium ions. Thus nitrogen assimilation via glutamate dehydrogenase (NADH-GDH) route is a bypass route when the normal GS-GOGAT route is inhibited. Results related to aminating activity of GDH which were recorded after 24 h and 72 h of experiments in both the cyanobacteria have been depicted in Fig. 18. Contrary to NR, NiR, GS and GOGAT activities, GDH activity exhibited enhanced activity under both the doses of CP and high dose of UV-B (UV-B<sub>H</sub>) treatments. An increase of 23 and 45% was noticed in *N. muscorum* and 12 and 32% increase was seen in the case of *P. foveolarum* after 24 h of treatments following CP<sub>L</sub> and CP<sub>H</sub> exposures, respectively. In the case of CP<sub>L</sub> dose, after 24 h, the primary route for ammonium assimilation (GS-GOGAT route) appeared to get restored hence lowered GDH activity was recorded. Further, over the period, GDH activity varied considerably suggesting that the stress, if any, was eased by the passage of time. After 72
of treatments, CP$_H$ and its different combinations persisted the increase in GDH activity, however CP$_L$ and its combinations showed inhibition in GDH activity.

3.5 Activities of enzymes related with phosphate metabolism

The role of nutrients, especially phosphorus as growth limiting factors in fresh water environment has gained much attention. Cyanobacteria assimilate phosphate at a much faster rate and accumulate large amounts of phosphate as reserve phosphate for an extended growth period at low phosphate concentrations. The characteristic feature of cyanobacteria is the production of phosphatases which are also common with plant roots and microorganisms in their microenvironment. When the level of phosphate declines, the cyanobacterial cells induce formation of alkaline phosphatase (ALP; in periplasm) and acid phosphatase (ACP; in cytoplasm) and their activity provide soluble form of phosphate (Pi) for the growth of cyanobacteria. Thus, due to the important roles of ALP and ACP in phosphate homeostasis, the impact of low and high fluence rate of UV-B on ALP and ACP activity was studied in both the cyanobacteria under low and high doses of chlorpyrifos.

3.5.1 Alkaline phosphatase activity

The ALP activity was estimated in the cells of *N. muscorum* and *P. foveolarum* after 24 and 72 h of experiments. The cells were centrifuged and resuspended in 0.2 M Tris-HCl buffer (pH 8.5) for 30 min. The reaction was initiated by adding 5.2 mM p-NP. The activity was measured in terms of nmol p-NP (mg protein)$^{-1}$ min$^{-1}$. One unit of ALP activity may be defined as 1 nmol p-nitrophenol produced min$^{-1}$. The results pertaining to ALP activity have been depicted in Fig. 19. Alkaline phosphatase activity exhibited an increase in its activity following UV-B$_L$, CP$_L$ or CP$_H$ treatments individually. CP$_L$ was able to raise the ALP activity by 27% in *N. muscorum* and by 18% in *P. foveolarum*, after 24 h of experiment, while CP$_H$ could cause much increment, by 58% in *N. muscorum* and by 30% in *P. foveolarum*, respective controls being 7.20±0.21 and 16.40±0.47 U mg$^{-1}$ protein. After 72 h of experiment, more or less reduction was recorded in ALP activities in all the samples except for UV-B$_H$, which after initial induction of 4% in *N. muscorum*
further improved, after 72 h of experiment. Whenever low dose of UV-B combined any of the CP doses, an enhanced ALP activity was obtained. Contrary to UV-B$_L$, when UV-B$_H$ is given along with any of the CP doses, ALP activities was lowered appreciably.

3.5.2 Acid phosphatase activity

The activity of ACP in *N. muscorum* and *P. foveolarum* was estimated after 24 and 72 h of experiments and the data have been portrayed in Fig. 20. The ACP activity in non-stressed (control) cells of *N. muscorum* and *P. foveolarum* was $6.10 \pm 0.18$ and $3.50 \pm 0.10$ U mg$^{-1}$ protein after 24 h, and $9.20 \pm 0.27$ and $5.30 \pm 0.15$ U mg$^{-1}$ protein after 72 h of experiments, respectively. CP$_L$ and CP$_H$ increased ACP activity by 37 and 63% in *N. muscorum* and by 34 and 27%, respectively in *P. foveolarum* after 24 h of experiment. Contrary to CP doses, UV-B$_H$ depressed ACP activity by 70% in *N. muscorum* and by 34% in *P. foveolarum* after 24 h of experiment. While, UV-B$_L$ was found to be insignificantly stimulatory, when it combined any of the CP doses, further stimulated the ACP activity. On the other hand UV-B$_H$ decreased the ACP activity vigorously when combined with any of the CP doses. Under similar conditions, ACP activity exhibited restoration after 72 h of experiments.

(B) Oxidative stress and antioxidative responses

3.6. Oxidative stress: generation of reactive oxygen species, oxidative damage and antioxidant system

3.6.1 Reactive oxygen species

A common feature of all stressed cells by which they respond is generation of reactive oxygen species (ROS). ROS are generated during metabolic processes. Under normal physiological conditions, the level of ROS is under the tight control of antioxidant defense system and thus they act as signaling molecules for evoking several useful processes such as lignin biosynthesis, gravitropic responses *etc.* in higher plants. However, under severe stress conditions, the generation of ROS accelerated and when it surpasses the antioxidant ability of organisms, causes severe damage to macromolecules
such as lipids, proteins and nucleic acids. In gerontology, generation of ROS is regarded as one of the important reasons for ageing. Thus, considering important role of ROS in physiology and cell biology of organisms including cyanobacteria, the impact of low and high fluence rates of UV-B on the generation of ROS i.e. SOR and H$_2$O$_2$ and their consequent damages to lipids and proteins have been investigated in the test cyanobacteria when they were simultaneously treated with low and high doses of chlorpyrifos.

3.6.1.1 Superoxide radical (SOR, O$_2^{•-}$)

Superoxide radical is generated in photosynthetic as well as respiratory electron transport reactions as intermediates of O$_2$ reduction. SOR has an unpaired electron that renders it highly reactive with biomolecules. SOR is short lived radical and negatively charged thus it does not diffuse through plasma membrane. Superoxide dismutase readily acts upon SOR and H$_2$O$_2$ is generated which is another damaging ROS.

The control cells showed 4.20±0.12 and 4.90±0.14 µmol SOR g$^{-1}$ DW content in $N$. muscorum and 2.70±0.08 and 3.00±0.09 µmol SOR g$^{-1}$ DW content in $P$. foveolarum after 24 and 72 h of experiments, respectively (Fig. 21). The figure shows that CP at tested doses (1 and 2 µg ml$^{-1}$) increased SOR content in both the organisms but more in $N$. muscorum. Treatment with CP$_L$ and CP$_H$ doses increased SOR content by 81 and 117% in $N$. muscorum, respectively after 24 h of experiment. Under similar condition, SOR content was increased by 56 and 88% in $P$. foveolarum, respectively after 24 h of experiment. However, the cyanobacterial samples exhibited appreciable lowering in the contents of SOR after 72 h of experiment than those recorded after 24 h of experiment. UV-B$_H$ dose also significantly accelerated SOR content in both the organisms. After 24 h of experiment, UV-B$_H$ accelerated SOR content by 102% in $N$. muscorum but only by 47% in $P$. foveolarum. After 72 of experiment, an appreciable lowering in SOR content was recorded in $N$. muscorum only, while $P$. foveolarum scored further enhancements in SOR content. Further, combined treatment of CP$_L$/CP$_H$ with UV-B$_H$ increased enhancements in SOR content in $N$. muscorum after 24 of experiments which later underwent a sharp decline. In the case of $P$. foveolarum, UV-B$_H$ reduced CP$_L$ induced
enhancement in SOR, while it enhanced that of CP₄ induced increase. On the other hand, UV-B₄ alone could not be able to raise the SOR content significantly, but when it combined with CP₄ dose treated \textit{N. muscorum} cells, it further increased SOR content, after 24 h of experiment. However, when measured after 72 h, a considerable decline of 16% in CP₄+UV-B₄ treated sample was observed in comparison to CP₄ alone. In the case of \textit{P. foveolarum}, UV-B₄ declined CP₄ as well as CP₄ induced enhancements after 24 h of experiment and further diminished per cent values were noticed when measured after 72 h.

3.6.1.2 Hydrogen peroxide (H₂O₂)

Hydrogen peroxide is formed either through the dismutation of SOR by the activity of enzyme superoxide dismutase or from other sources. Results pertaining to H₂O₂ content in tested cyanobacteria under low and high doses of chlorpyrifos and also under low and high doses of UV-B alone as well as in combinations have been depicted in Fig. 22. Control cells exhibited 9.20±0.27 and 9.80±0.28 µmol H₂O₂ g⁻¹ DW in \textit{N. muscorum} and 6.60±0.19 and 6.90±0.20 µmol H₂O₂ g⁻¹ DW in \textit{P. foveolarum} after 24 and 72 h of experiments, respectively. Results revealed that cells treated with CP₄ and CP₄ accelerated H₂O₂ production by 56 and 95% in \textit{N. muscorum} and 35 and 78% in \textit{P. foveolarum}, respectively after 24 h of experiment. However, under similar treatments, an appreciable reduction in per cent H₂O₂ content was recorded after 72 h of experiment as compared to the results of 24 h. Similarly, UV-B₄ enhanced H₂O₂ production by 86% in \textit{N. muscorum} and by 45% in \textit{P. foveolarum} after 24 h of experiment, however, after 72 h of experiment, the H₂O₂ content increased only by 40% in \textit{N. muscorum} but by 80% in \textit{P. foveolarum}. Combined treatment of CP and UV-B₄ further enhanced H₂O₂ production than their individual treatment. Maximum production of H₂O₂ was recorded in \textit{N. muscorum} under the treatment of CP₄+UV-B₄ after 24 h of experiment. In contrast to UV-B₄, UV-B₄ dose alone did not significantly influence H₂O₂ content in tested cyanobacteria. Furthermore, when UV-B₄ dose was given together with any of the CP doses, significantly lowered H₂O₂ content in both the organisms in comparison to the CP treatments alone.
3.6.2 Oxidative damage indices

Cellular membranes are made up of polyunsaturated fatty acids (PUFA) and proteins and perform different functions. The lipids and proteins are highly susceptible to reactive oxygen species (O$_2^\cdot$·, H$_2$O$_2$, ·OH etc.) formed in excess during various metabolic processes in response to stresses such as pesticides and UV-B. In a cell, oxidative damage is manifested into elevated concentration of oxidatively modified macromolecules such as lipids and proteins. Lipid peroxidation considerably increases permeability of cell membranes leading to the alterations in the physiological functions and consequently to cellular damage. Protein oxidation or carbonylation involves the modification of side chains of certain amino acids resulting into production of ketone or aldehyde derivatives. Formation of carbonyl groups alters the biological role of proteins and in the case of enzymes, it often leads to the inhibition of their activity. Protein carbonylation, due to its irreversibility, is considered to be one of the most serious modifications of the cell macromolecules. The assessment of lipid peroxidation product malondialdehyde (MDA) and protein oxidation (reactive carbonyl groups) can provide the impression of the extent of oxidative damage in *N. muscorum* and *P. foveolarum* exposed to CP and UV-B$_H$. Thus, lipid peroxidation and protein oxidation are considered as important indices for assessing the damage caused by various stresses.

3.6.2.1 Lipid peroxidation– MDA formation

ROS mediated lipid peroxidation was measured in terms of malondialdehyde (MDA) accumulation in tested cyanobacteria exposed to low and high doses of CP and UV-B radiation after 24 and 72 h of experiments. Results depicted in Fig. 23 show that MDA accumulation was higher in *N. muscorum* than *P. foveolarum* when exposed to either doses of CP or UV-B$_H$. The amount of MDA in control cells was 0.91±0.03 and 0.95±0.03 μmol MDA g$^{-1}$ DW in *N. muscorum* and 0.54±0.02 and 0.61±0.02 μmol MDA g$^{-1}$ DW in *P. foveolarum* after 24 and 72 h of experiments, respectively. Further, results revealed that CP$_L$ and CP$_H$ doses accelerated MDA accumulation by 31 and 75% in *N. muscorum*, respectively and by 22 and 60%, respectively in *P. foveolarum* after 24 h of
experiment. However, under similar treatments, after 72 h of experiment, though MDA accumulation in tested cyanobacteria was higher than those of control cells but their levels were appreciably lowered than those recorded after 24 h of experiment. Similar to CP treatments, UV-B\textsubscript{H} dose also significantly enhanced MDA accumulation in both the cyanobacteria as it was increased by 76% in \textit{N. muscorum} and by 70% in \textit{P. foveolarum} after 24 h of experiment, however, after 72 h of experiment lowering in MDA accumulation was observed. Further, results revealed that under combined treatments of CP\textsubscript{L}/CP\textsubscript{H} with UV-B\textsubscript{H}, the MDA accumulation exacerbated than their individual treatments. After 24 h of experiment, treatments of CP\textsubscript{L}+UV-B\textsubscript{H} and CP\textsubscript{H}+UV-B\textsubscript{H} accelerated MDA accumulation by 67 and 106% in \textit{N. muscorum} and by 78 and 138% in \textit{P. foveolarum}, respectively. However, after 72 h of experiment, under similar treatments, an appreciable decline in MDA level over the values of 24 h of experiment was noticed in tested cyanobacteria. In contrast to UV-B\textsubscript{H}, UV-B\textsubscript{L} dose of UV-B radiation alone did not significantly influence MDA level in both the cyanobacteria. Furthermore, it was observed that when UV-B\textsubscript{L} dose was given in combination of any of the CP doses, it significantly lowered CP-induced accumulation of MDA in tested cyanobacteria.

\textbf{3.6.2.2 Protein oxidation– reactive carbonyl group (RCG, >C=O) formation}

Protein oxidation is another indicator of oxidative damage, determined in terms of reactive carbonyl group (RCG) formation in both the cyanobacteria exposed to CP and UV-B. The untreated cells experienced 2.00±0.06 and 2.30±0.07 \textmu mol RCG g\textsuperscript{-1} DW in \textit{N. muscorum} and 1.50±.04 and 1.70±0.05 \textmu mol RCG g\textsuperscript{-1} DW in \textit{P. foveolarum} after 24 and 72 h of experiments, respectively (Fig. 24). The results showed that CP\textsubscript{L} and CP\textsubscript{H} doses raised RCG content by 21 and 70%, respectively in \textit{N. muscorum} after 24 h of experiment. Under similar treatments, in \textit{P. foveolarum}, RCG content was increased by 19 and 50%, respectively after 24 h of experiment. Exposure of UV-B\textsubscript{H} dose enhanced RCG content by 160% in \textit{N. muscorum} and by 112% in \textit{P. foveolarum}. Further, UV-B\textsubscript{H} together with any of the CP doses accelerated RCG formation compared to their individual treatments. Cells treated with CP\textsubscript{L}+UV-B\textsubscript{H} and CP\textsubscript{H}+UV-B\textsubscript{H} treatments showed an increment in RCG by 180 and 196% in \textit{N. muscorum} and by 131 and 151% in
Experimental Findings

3.6.3 Antioxidant system

Mechanisms to keep the ROS level under limit during stress are crucial for all aerobic organisms. Cyanobacteria have developed numerous strategies in order to scavenge ROS. Cyanobacteria express a wide array of antioxidants to mitigate ROS induced damage to macromolecules. Antioxidants are of two types: enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), peroxidases *i.e.* guaiacol peroxidase (POD), glutathione peroxidase (GPX) *etc.* Ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) also play important role in indirect mitigation and management of ROS by providing reduced forms of glutathione and ascorbate. Non-enzymatic antioxidants such as ascorbate, glutathione, non-protein thiols [NP-SH] and cysteine also play major role in indirect and direct scavenging of ROS. The importance of enzymatic and non-enzymatic antioxidants in preventing oxidative stress in cyanobacteria is based on the fact that level of one or more antioxidants increases under stress which is generally related with increased stress tolerance. Thus, considering their important roles in mitigating negative consequence of ROS, the impact of low and high doses of UV-B radiation on antioxidative capacity of both the cyanobacteria under low and high doses of chlorpyrifos has been investigated in detail.

3.6.3.1 Enzymatic antioxidants

3.6.3.1.1 Superoxide dismutase activity

SOD is a ubiquitous metalloenzyme that is found in organisms having oxygen metabolism. SOD catalyzes disproportionation of highly reactive and damaging $O_2^{•-}$ into comparatively less damaging $H_2O_2$.

$$2O_2^{•-} + 2H^+ \xrightarrow{\text{SOD}} 2H_2O_2 + O_2$$
Thus, SOD represents first line of defense against ROS. SOD activity in tested cyanobacteria exposed to low and high doses of UV-B radiation under low and high doses of chlorpyrifos was measured after 24 h and 72 h of experiments and results have been presented in Tables 7 and 8. Results revealed that exposure of both the cyanobacteria to CP_L resulted into an increased activity of SOD after 24 h as well as 72 h of experiments. Results showed that treatments of CP_L accelerated SOD activity by 23 and 30% in *N. muscorum* and *P. foveolarum*, respectively after 24 h of experiment. The same cyanobacterial samples showed an increase in SOD activity compared to control samples, after 72 h of experiment. In case of UV-B_H exposure, there were enhancements in SOD activity by 18 and 22% in *N. muscorum* and by 9 and 25% in *P. foveolarum* after 24 h and 72 h of experiments, respectively. On combining UV-B_L dose with any of the CP doses, the SOD activity was increased, and CP_H+UV-B_L treated sample of *N. muscorum* exhibited 9% more SOD activity while that of *P. foveolarum* exhibited 6% more SOD activity than CP_H treatment alone when measured after 24 h. While CP_H alone was always proved to be inhibitory on SOD activity of both the tested cyanobacteria, inhibited values did recover when measured after 72 h of experiments. Maximum stimulation in SOD activity (by 50%) was noticed under CP_L+UV-B_L treatment in *P. foveolarum* after 24 h of experiment. UV-B_L dose alone as well as together with both the doses of CP showed stimulation in SOD activity.

### 3.6.3.1.2 Catalase activity

H₂O₂ has been implicated in many stress conditions. CAT scavenges H₂O₂ and converts it into H₂O and O₂ and thus avoids occurrence of oxidative stress in cell. CAT activity does not require reducing power for dissociation of H₂O₂ and has a high reaction rate with poor affinity with H₂O₂. Results pertaining to CAT activity in both the cyanobacteria exposed to two doses of CP along with the two doses of UV-B after 24 h and 72 h of experiments are shown in Tables 7 and 8. CAT activity was recorded 10.50±0.30 and 13.30±0.38 U mg⁻¹ protein in control cells of *N. muscorum* and 16.00±0.46 and 21.50±0.62 U mg⁻¹ protein in control cells of *P. foveolarum* after 24 and 72 h of experiments, respectively. CP_L enhanced CAT activity by 50% in *N. muscorum*
and by 32% in *P. foveolarum*, respectively after 24 h of experiment. After 72 h of experiment, under similar treatments, increase in CAT activity remained only 32% in *N. muscorum* and 21% in *P. foveolarum*, respectively. *Nostoc muscorum* experienced reduction in CAT activity when treated with CP$_H$ after 24 h of experiment, and improved by meager 3% after 72 h in comparison to control sample, while *P. foveolarum* had 6% more activity. On the other side, UV-B$_H$ significantly inhibited CAT activity as it was declined by 24% in *N. muscorum* and by 20% in *P. foveolarum* after 24 h of experiment. However, after 72 h of experiment, recovery in CAT activities was observed in UV-B$_H$ exposed cyanobacterial samples. When chlorpyrifos supplemented cyanobacterial samples additionally treated with UV-B$_H$, CAT activity substantially lowered in comparison to CP treatments alone but still CP$_L$+UV-B$_H$ treated cells had 17% more CAT activity in *N. muscorum* cells and 30% more CAT activity in *P. foveolarum* when assayed after 24 h, and 6% more CAT activity in *N. muscorum* and 28% more CAT activity in *P. foveolarum* after 72 h of experiment. However, when UV-B$_H$ was given in addition to CP$_H$, the CAT activity was decreased by 40% in *N. muscorum* and by 25% in *P. foveolarum*, after 24 h of experiment, but the same samples showed appreciable recovery after 72 h of experiment. In contrary to UV-B$_H$ dose, UV-B$_L$ radiation alone significantly stimulated CAT activity as it was enhanced by 9 and 8% in *N. muscorum* and 12 and 9% in *P. foveolarum* after 24 and 72 h of experiments, respectively (Tables 7 and 8). Furthermore, when UV-B$_L$ was given in combination with CP$_L$, it further enhanced CP$_L$ induced enhancements in CAT activity of both the test cyanobacteria, and prevented the inhibitions in CP$_H$ treated cells.

### 3.6.3.1.3 Peroxidase activity

Peroxidase (POD) also decomposes H$_2$O$_2$ into H$_2$O and O$_2$ and thus plays an important role in averting oxidative stress. In the present study, pyrogallol was used to assess the POD level in treated and untreated cyanobacteria. Results related to POD activity in both the cyanobacteria have been shown in Tables 7 and 8. Results revealed that CP$_L$ and CP$_H$ doses were able to raise the POD level with different extent in both the cyanobacteria. CP$_L$ and CP$_H$ enabled *N. muscorum* to raise its POD level by 24 and 52%
after 24 h of experiment, while these treatments enabled *P. foveolarum* to raise POD activity by 35 and 73% in same duration in comparison to its respective controls. These values after 72 h of experiment, came to a lower per cent value in comparison to their respective controls. Low dose of UV-B more or less significantly raised the POD level in *N. muscorum* as well as in *P. foveolarum* cells. On the other side, UV-B<sub>H</sub> increased the POD level quite high in both the tested cyanobacteria. In addition to the above, whenever UV-B<sub>L</sub> or UV-B<sub>H</sub> combined any of the CP doses in both the cyanobacteria, it raised the respective POD values. Further, when 72 h POD was measured, the per cent value of each and every sample diminished when compared to their 24 h allies.

### 3.6.3.1.4 Glutathione-S-transferase activity

GST removes xenobiotics (e.g. pesticides, antibiotics etc.), metals and lipid peroxidation and protein oxidation products (which may further initiate free radical chain reaction) using reduced glutathione. Thus, GST plays crucial role for survival of organisms during stress conditions. The GST activity in tested cyanobacteria exposed to low and high doses of UV-B under low and high doses of chlorpyrifos was investigated after 24 as well as 72 h of experiments and results are presented in Tables 7 and 8. The GST activity was recorded 34.00±0.98 and 48.00±1.39 U mg<sup>-1</sup> protein in untreated cells of *N. muscorum* and 22.80±0.66 and 27.50±0.79 U mg<sup>-1</sup> protein in control cells of *P. foveolarum* after 24 and 72 h of experiments, respectively. CP<sub>L</sub> dose enhanced GST activity by 15% in *N. muscorum* and by 27% in *P. foveolarum* when recorded after 24 h in comparison to the control cells. After 72 h, the activity remained 7% more in *N. muscorum* and 21% more in *P. foveolarum* cells, compared to their respective untreated control cells. CP<sub>H</sub> seemed to be initially inhibitory for the GST activity of *N. muscorum* which could raise the activity in *N. muscorum* only after 72 h, however CP<sub>H</sub> induced increments in GST activity in *P. foveolarum* was noticed from very beginning. Low dose of UV-B more or less insignificantly raised the GST activity, but when it joined with any of the two insecticidal doses, there was increased GST activity. On the other hand, UV-B<sub>H</sub> also raised the GST activity and further enhanced the CP<sub>L</sub> and CP<sub>H</sub> altered GST activities (except in CP<sub>H</sub> treated *N. muscorum* cells, where simultaneous exposure of UV-B<sub>H</sub> decreased the existing GST activity).
3.6.3.1.5 Ascorbate peroxidase activity

The accumulation of $\text{H}_2\text{O}_2$ is also prevented by APX. APX is an important enzyme of ascorbate-glutathione cycle and scavenges $\text{H}_2\text{O}_2$ using ascorbate as electron donor. Results revealed that *P. foveolarum* exhibited higher amount of APX activity than *N. muscorum* as APX activity in control cells of *P. foveolarum* was $2750.00\pm79.00$ U mg$^{-1}$ protein and in *N. muscorum*, it was $1365.00\pm39.00$ U mg$^{-1}$ protein after 24 h of experiment (Tables 9 and 10). When the two cyanobacteria were treated with CP$_L$, they exhibited significant enhancements in APX activity as it was enhanced by 24 and 39% in *N. muscorum* and *P. foveolarum*, respectively after 24 h of experiment. On the other hand, when both organisms treated with CP$_H$ dose, declined the APX activity by 29% in *N. muscorum* and by 19% in *P. foveolarum* after 24 h of experiment. However, after 72 h of experiments, under CP$_L$ treatments, the increase in APX activity was 23% in *N. muscorum* and 29% in *P. foveolarum* but under CP$_H$ treatment decrease was recorded by 16% in *N. muscorum* and by 6% in *P. foveolarum*, respectively after 72 h of experiment. Similar to CP$_H$ treatments, UV-B$_H$ dose also declined APX activity by 31% in *N. muscorum* and by 24% in *P. foveolarum*, after 24 h of experiment while it reduced the APX activity by 24% in *N. muscorum* cells and by 17% in *P. foveolarum* cells, after 72 h of experiment. Results show that when both the organisms were exposed to UV-B$_L$, insignificant stimulation in APX activity was seen, however it was successful to enhance the CP$_L$ or CP$_H$ induced alterations in APX activities.

3.6.3.1.6 Glutathione reductase activity

GR is also an important enzyme of ascorbate-glutathione cycle. GR is a member of flavoenzyme family, catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) into reduced glutathione (GSH). This reaction is essential for maintenance of a proper GSH/GSSG ratio in cells which is believed to be useful for mitigating the ROS induced damage in cells. Dehydroascorbate reductase (DHAR) catalyzes reduction of dehydroascorbate (DHA) into reduced ascorbate (AsA) using GSH as electron donor and GSH is converted into GSSG. Further, it is well known that APX uses AsA as electron donor for the dissociation of $\text{H}_2\text{O}_2$. Thus, reduction of GSSG into
GSH by GR becomes essential for further scavenging of $\text{H}_2\text{O}_2$ by APX during stress conditions.

GR activity in both the cyanobacteria exposed to low and high doses of UV-B under low and high CP treatments was investigated after 24 h as well as 72 h of experiments and the results have been shown in Tables 9 and 10. Results showed that untreated cells of *P. foveolarum* contained more GR activity than those of *N. muscorum*. The GR activity was recorded 17.00±0.49 and 22.50±0.65 U mg$^{-1}$ protein in the control cells of *N. muscorum* and 25.00±0.72 and 30.00±0.87 U mg$^{-1}$ protein in the control cells *P. foveolarum* after 24 and 72 h of experiments, respectively. Results also reveal that inherent level of GR in control cells was higher when recorded after 72 h of experiment than those recorded after 24 h of experiment. Both the cyanobacteria exhibited differential alterations in GR activity when treated differentially in various combinations of low and high doses of CP and UV-B. GR increased after CP$_L$ dose treatment. UV-B$_L$ treated cyanobacteria showed some signs of enhancement, and when UV-B$_L$ was given together with CP$_L$, higher enzyme activity was recorded than those recorded for CP$_L$ dose alone. Further, when UV-B$_L$ was supplemented with CP$_H$ dose, considerable improvement in GR activity was noticed. Contrary to the above, GR activity got significantly reduced when either treated with CP$_H$ dose or exposed to UV-B$_H$. The reductions got aggravated when CP$_H$ was given together with UV-B$_H$. But, when either UV-B$_H$ was given along with CP$_L$ or UV-B$_L$ was given along with CP$_H$, GR activity was found to be higher than those found for UV-B$_H$ or CP$_H$ treatments alone. Thus, UV-B$_L$ and CP$_L$ always posed a positive impact on GR activity. After 72 h of experiments, GR activity of different samples showed a strong sign of recovery when compared with those of 24 h (except UV-B$_H$ treated *N. muscorum* cells). UV-B$_L$ alone increased GR activity insignificantly, but together with CP$_H$ dose, GR activity showed considerable improvement.

### 3.6.3.1.7 Monodehydroascorbate reductase activity

One of the key enzymes in ascorbate-glutathione cycle is monodehydroascorbate reductase (MDHAR), an FAD enzyme that catalyses the reduction of
monodehydroascorbate radical to regenerate ascorbate which is further used by APX to detoxify H$_2$O$_2$. Results indicated that MDHAR activity was greater in $P.$ foveolarum than in $N.$ muscorum (Tables 9 and 10). Control cells of $N.$ muscorum exhibited 98.00±2.85 U mg$^{-1}$ protein after 24 h of experiment, while those of $P.$ foveolarum exhibited 122.50±3.54 U mg$^{-1}$ protein. These values increased up to the level of 117.60±3.39 U mg$^{-1}$ protein in $N.$ muscorum and 151.40±4.37 U mg$^{-1}$ protein in $P.$ foveolarum after 72 h. CP$_L$ enhanced MDHAR activity by 32% in $N.$ muscorum and by 41% in $P.$ foveolarum, after 24 h of experiment. CP$_H$, however, reduced the MDHAR values significantly compared to respective controls. When CP$_L$ dose was supplemented with UV-B$_L$, further enhanced DHAR activity, but lowering in per cent value was also observed over the period of 72 h. When CP$_H$ treated samples were supplemented with UV-B$_L$, though the CP$_H$ induced inhibition was lowered, but not effectively. UV-B$_H$ was inhibitory, but when it was exposed to CP$_L$ treated cells, the MDHAR activity was increased considerably. But, this did not happen with CP$_H$ +UV-B$_H$ samples.

3.6.3.1.8 Dehydroascorbate reductase activity

DHAR is also an important enzyme of AsA-GSH cycle. APX uses AsA as electron donor for scavenging H$_2$O$_2$ and converts AsA into monodehydroascorbate (a single electron oxidation product). Two molecules of monodehydroascorbate undergo the process of disproportionation forming one molecule of AsA and one molecule of dehydroascorbate (a double electron oxidation product). Dehydroascorbate is reduced into AsA by the action of DHAR which uses GSH as electron donor. Thus, DHAR maintains AsA/DHA ratio in cells under stressed conditions for mitigating the negative consequences of ROS on macromolecules.

Results indicated that DHAR activity was also greater in $P.$ foveolarum than in $N.$ muscorum (Tables 9 and 10). Control cells of $N.$ muscorum exhibited 430.00±12.41 U mg$^{-1}$ protein after 24 h of experiment, while those of $P.$ foveolarum exhibited 513.00±14.81 U mg$^{-1}$ protein. These values were increased up to the level of
563.00±16.25 U mg\(^{-1}\) protein in \(N.\) muscorum and 700.00±20.21 U mg\(^{-1}\) protein in \(P.\) foveolarum after 72 h. \(CPL\) enhanced DHAR activity by 12\% in \(N.\) muscorum and by 22\% in \(P.\) foveolarum, after 24 h of experiment. However, \(CPH\) reduced the DHAR activity significantly compared to respective controls. Low dose of UV-B\(_L\) insignificantly raised the activity of DHAR in \(N.\) muscorum, but significant stimulation was seen in \(P.\) foveolarum cells, after 24 h. When either of the CP doses was supplemented with UV-B\(_L\), DHAR activity was enhanced, but lowering in value was also observed over the period of 72 h. The \(CPH\) induced inhibitions were alleviated when \(CPH\) dose was supplemented with UV-B\(_L\). UV-B\(_H\) was inhibitory, but when supplemented with \(CPL\) dose, the DHAR activity was dramatically increased. Again, this did not happen with \(CPH+UV-BH\) samples.

### 3.6.3.2 Non-enzymatic antioxidants

Apart from enzymatic antioxidants, non-enzymatic antioxidants \textit{i.e.} ascorbate, glutathione, non-protein thiols (NP-SH) and cysteine also play crucial role in direct or indirect scavenging of ROS. Thus, considering their important roles in mitigating negative consequence of ROS, in the present study, the impact of low and high doses of UV-B on non-enzymatic antioxidant capacity of both the cyanobacteria singly and simultaneously exposed to chlorpyrifos has also been investigated in detail.

#### 3.6.3.2.1 Total ascorbate and reduced to oxidized ascorbate ratio

Ascorbate is a crucial non-enzymatic antioxidant involved in defense against oxidative stress. Ascorbate reacts with \(^1O_2\), \(O_2^-\), and \(^*\)OH, and acts as the natural substrate of many plant peroxidases. Thus, due to its important role in scavenging ROS, the impact of low and high doses of UV-B radiation on total ascorbate (AsA+DHA), reduced ascorbate (AsA), dehydroascorbate (DHA) and AsA/DHA ratio was investigated in both the cyanobacteria under low and high doses of chlorpyrifos after 24 h as well as 72 of experiments.

Results pertaining to total ascorbate level and the ratio of reduced/oxidised
ascorbate of *N. muscorum* and *P. foveolarum* have been shown in Tables 11 and 12, respectively. Results revealed that untreated cells contained 50.40±1.45 and 87.00±2.51 µmol g⁻¹ DW in *N. muscorum* and 79.50±2.3 and 132.40±3.82 µmol g⁻¹ DW in *P. foveolarum* after 24 and 72 h of experiments, respectively. Thus, control cells of *P. foveolarum* contained more ascorbate than *N. muscorum*. Results showed that the AsA+DHA and the ratio of AsA/DHA were decreased by CP₇ and UV-B₇ treatments. Results reveal that after 72 h of exposure of CP₇ dose of the insecticide, there occurs a shift from oxidized to reduced form of ascorbate. But, at the same time, with CP₇ dose, oxidized form of ascorbate was found greater than those obtained after 24 h, and thus lower reduced/oxidized ratio was obtained. In the same way, UV-B₇ also enhanced the reduced form of ascorbate while higher amount of oxidized ascorbate with UV-B₇ dose was recorded after 24 h of exposure. When CP₇ and CP₇ doses were supplemented with UV-B₇ dose, later played its recovery role and reduced/oxidized ratio increased while exactly reverse happened with UV-B₇. After 72 h, the amount of reduced ascorbate increased in comparison to the values estimated after 24 h but for severely stressed cells like CP₇, UV-B₇, CP₇+UV-B₇, CP₇+UV-B₇, CP₇+UV-B₇, the values of oxidized ascorbate was still higher than the respective controls. A sharp decrease in total ascorbate ensued with CP₇ dose alone and also with CP₇+UV-B₇ but the amount tended to recover after 72 h of experiment.

### 3.6.3.2.2 Total glutathione and reduced to oxidized glutathione ratio

Glutathione is considered as one of the most important and abundant cytosolic components of the non-enzymatic antioxidant system, being present at high intracellular concentrations. Its function includes ROS scavenging before damaging reactive molecules initiate their chain reaction-damaging effects on macromolecules. Thus, considering the important role of GSH in ROS scavenging processes, the impact of low and high doses of UV-B radiation on total glutathione (GSH+GSSG), reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG ratio was investigated in both cyanobacteria under low and high doses of CP after 24 and 72 h of experiments.
Results related to GSH+GSSG and GSH/GSSG are shown in Tables 11 and 12. Similar to ascorbate, CP$_H$ and UV-B$_H$ produced significant decline in GSH+GSSG, GSH and GSH/GSSG ratio in both the cyanobacteria. However, glutathione content was affected more readily in N. muscorum than P. foveolarum. The GSH+GSSG was recorded as 28.70±0.83 and 39.50±1.14 µmol g$^{-1}$ DW in N. muscorum and P. foveolarum after 24 h, and 43.37±1.25 and 52.70±1.52 µmol g$^{-1}$ DW in N. muscorum and P. foveolarum, respectively after 72 h of experiment. CP$_L$ exposure resulted into increase in GSH+GSSG by 24% in N. muscorum and 18% in P. foveolarum while CP$_H$ declined total glutathione content by 10% in N. muscorum and by 7% in P. foveolarum, respectively after 24 h of experiment. However, under similar treatments, after 72 h of experiment, decrease in per cent content of GSH+GSSG was recorded in case of CP$_L$ dose, while a recovery was recorded in CP$_H$ treated cells in comparison to their control cells. UV-B$_H$ dose alone substantially declined GSH+GSSG content and GSH/GSSG ratio in both the cyanobacteria. Under UV-B$_H$ exposure, GSH+GSSG content decreased by 43% in N. muscorum and by 51% in P. foveolarum, after 24 h of experiment. However, after 72 h of experiment, considerable improvement in GSH+GSSG and GSH/GSSG ratio was noticed in the cells exposed to UV-B$_H$. Combined treatments of CP$_H$ and UV-B$_H$ produced rapid decrease in glutathione content in N. muscorum when compared with their individual treatments. After 24 h of experiments, treatments of cyanobacterial cells with CP$_H$+UV-B$_H$ led to a decrease in GSH+GSSG content by 44 and 42% in N. muscorum and P. foveolarum, respectively. However after 72 h of experiment, under similar treatments, restoration trend was seen in GSH+GSSG and GSH contents. Unlike UV-B$_H$ dose, when UV-B$_L$ dose was given together with CP$_L$, it further enhanced GSH+GSSG contents and GSH/GSSG ratio, while it appeared to restore the lost GSH+GSSG content under CP$_H$ dose after 72 h of experiment.

3.6.3.2.3 Cysteine

Cysteine is an amino acid having thiol side chain, often participates in enzymatic reactions serving as nucleophile. The thiol of cysteine is susceptible to oxidation to give disulfide derivative, which serves an important structural role in many proteins. Cysteine also participates in the detoxification of xenobiotics and thus it can lead to resistance
against pesticide stress. Cysteine content in both untreated and treated cells of cyanobacteria exposed to low and high levels of UV-B was measured after 24 h as well as 72 h of experiments and results have been depicted in Fig. 25. The figure shows that the inherent level of cysteine in the control cells of *P. foveolarum* was more than the level of *N. muscorum*. The cysteine content was 4.97±0.14 and 3.60±0.10 µmol g^-1 DW in control cells of *N. muscorum* and 19.20±0.55 and 12.70±0.37 µmol g^-1 DW in control cells of *P. foveolarum*, after 24 and 72 h of experiments, respectively. CP_L dose caused an increase in cysteine content in both the cyanobacteria after 24 h as well as 72 h of experiments, while CP_H caused reduction in cysteine level. Contrary to most of the results, cysteine content was drastically affected by UV-B_L in *P. foveolarum*. But, interestingly when UV-B_L combined with CP_L dose, the induction in cysteine content was recorded and CP_H dose induced decrease in cysteine content was recovered in the case of *N. muscorum* only. Being damaging, UV-B_H further declined CP_H induced reduction in cysteine level in both the cyanobacteria.

### 3.6.3.2.4 Non-protein thiols

Non-protein thiol (NP-SH) compounds can be found in most of the plants, microorganisms and all mammalian tissues. In several plant species, they exist in the form of γ-L-glutamylcysteinyl glycine. These compounds exist in the thiol-reduced and disulfide-oxidized forms. They play a key role in the regulation of the redox balance and can be regarded as an indicator of oxidative stress, act in the detoxification of xenobiotics, heavy metals and UV-B induced damages. Owing to their important role in mitigation of oxidative stress, the amount of NP-SH in both the cyanobacteria exposed to low and high UV-B radiations simultaneously exposed to low and high doses of chlorpyryrifos was determined after 24 h and 72 h of experiments.

Results pertaining to NP-SH level in tested cyanobacteria have been depicted in Fig. 26. The NP-SH content was recorded as 31.40±0.91 and 38.80±1.12 µmol g^-1 DW in *N. muscorum* and 252.50±7.29 and 275.30±7.95 µmol g^-1 DW in *P. foveolarum* after 24 and 72 h of experiments, respectively. Thus, results showed that control cells of *P.*
*P. foveolarum* contained several folds higher NP-SH than that of *N. muscorum*. After 24 h of experiment, *N. muscorum* cells showed an increment of 8% after CP<sub>L</sub> treatment and a decline of 9% under CP<sub>H</sub> toxicity. However, after 72 h of experiments, CP<sub>H</sub> treated *N. muscorum* cells showed some signs of recovery in NP-SH level. Under similar treatments and experimental conditions, 7 and 26% increase was seen in NP-SH level in *P. foveolarum*. UV-B<sub>L</sub> insignificantly posed a positive impact on NP-SH level. Under the simultaneous exposure of UV-B<sub>L</sub>, CP<sub>L</sub> induced enhancements in NP-SH level further got enhanced in both the cyanobacteria and CP<sub>H</sub> induced decrease was improved considerably in *N. muscorum*, after 24 as well as 72 h of experiments. On the other side, UV-B<sub>H</sub> having stimulatory impacts on NP-SH content after 72 h of experiments, also enhanced CP<sub>L</sub> induced enhancements in NP-SH level, but when UV-B<sub>H</sub> dose was given along with CP<sub>H</sub>, CP<sub>H</sub> induced reduction got further declined in *N. muscorum*, however NP-SH content enhanced appreciably in case of *P. foveolarum*. 