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Pretilachlor toxicity is decided by discrete photo-acclimatizing conditions: Physiological and biochemical evidence from *Anabaena* sp. and *Nostoc muscorum*

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ABSTRACT

The current study was undertaken to elucidate the impact of the herbicide pretilachlor (3 μg ml^{-1} and 6 μg ml^{-1}) on cyanobacteria, *Nostoc muscorum* ATCC 27893 and *Anabaena* sp. PCC 7120 under three levels of photoacclimatization (suboptimum, 25 μmol photon m^{-2} s^{-1}; optimum, 75 μmol photon m^{-2} s^{-1}; and supra-optimum, 225 μmol photon m^{-2} s^{-1}) by analyzing certain physiological (biomass accumulation, photosynthesis, Chl *a* fluorescence and respiration) and biochemical parameters (photosynthetic pigments– chlorophyll *a*, carotenoids and phycocyanin; reactive oxygen species– $O_2^{\bullet-}$, H_2O_2, lipid peroxidation; antioxidant system– superoxide dismutase, peroxidise, catalase and glutathione-*S*-transferase). The light conditioning played the most prominent role in deciding the extent of herbicide toxicity on both the tested cyanobacteria as the maximum toxicity was observed in suboptimum light acclimatized cyanobacterial cells corroborated by the least growth in the same cells. The impact of pretilachlor treatment on photosystem II photochemistry *viz.* φP_0, Ψ_0, φE_0, PI_{ABS}, ABS/RC, TR_0/RC, ET_0/RC *and* DI_0/RC was also altered by light acclimatization. The percent rise in oxidative stress markers (SOR and H_2O_2) and consequent lipid peroxidation (MDA equivalents) were also highest in suboptimum light acclimatized cells exposed to pretilachlor which could not be prospered with compatible antioxidant performance. Conversely, supra-optimum light acclimatized cells of both the cyanobacteria was found to accelerate the activities of all the studied enzymes and thus able to counterbalance the pretilachlor toxicity and supported the healthier growth.

1. Introduction

Solar light exhibits momentary, diurnal, seasonal and global variations on the earth surface in the terms of irradiance and spectral distribution. Further, along the water column in water bodies, both irradiance and light spectral composition depends upon the angle of the incidence, optical property (absorption and scattering processes), gilvin and tripton (dissolved and particulate organic matter) and planktons (Kirk, 1994). Photoautotrophs sense many traits of light in their environment, including intensity, wavelength, duration and direction. Thus, phytoplanktons are bound to perceive different amounts of light (photosynthetic photon flux density-PPFD) and survive accordingly.

Light is perhaps the most important biological factor which affects the basic physiological processes of plants such as photosynthesis, transpiration, seed germination, flowering *etc*. There must be a kind of light adaptation in plants' photosynthetic machinery including its light harvesting ability for electron flow and numerous enzymatic activities for perceiving certain amount of light intensity for a particular period (photoperiod) throughout the year.

Light intensity adaptations are usually characterized by modification in photosynthetic apparatus, mainly in light-harvesting components (Falkowski et al., 1981). The amount of two photosystems alters in response to altered light conditions in the chloroplasts of green algae and higher plants. The ultrastructure of chloroplast adapts to changing

Abbreviations: ABS/RC, The energy fluxes for absorption of photon per active reaction center; CAT, Catalase; DI_0/RC, Energy dissipation flux per active reaction center; ET_0/RC, Electron transport flux per active reaction center; *Fv/Fm* or ϕP_0, The quantum yield of primary photochemistry; GST, Glutathione-*S*-transferase; H_2O_2, Hydrogen peroxide; MDA, Malondialdehyde; ϕE_0, Quantum yield of electron transport; PI_{ABS}, Performance index of PSII; POD, Peroxidase; Ψ_0, Yield of electron transport per trapped exciton; *RC*, Reaction center; SOR, Superoxide radical; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TR_0/RC, Trapped energy flux per active reaction center

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light circumstances (Melis, 1984). For the instance, plants perceiving far-red or low light have more thylakoid membranes in their chloroplasts while the plants grown under blue or high light reduction in thylakoid membranes and decrease in the PS I/PS II ratio are noticed (Lichtenthaler et al., 1981). Long-term adaptations to excess of light lead to adjustments in light-harvesting antenna size in order to decrease light absorption and increase light utilization. Additionally, an orchestrated network of defence prospered by antioxidants (enzymes and metabolites like Vit C, glutathione, proline *etc*.) protects chloroplasts from excess light induced oxidative stress and let them to acclimatize rapidly to vagaries light conditions (Golan et al., 2004).

Cyanobacteria flourish in water-bodies where quality, intensity and duration of light vary considerably. Evidence show that cyanobacteria often use 70–80 μmol photon m^{-2} s^{-1} of PAR for their optimum growth and developmental process (Kilian et al., 2007). However, cyanobacteria receive only a fraction of solar light in paddy fields in submerged condition. Additionally, cyanobacteria may be a model photosynthetic organism to study various light adaptations as they resemble other algae and higher plants. Like higher plants, cyanobacteria do have strategies for the optimal use of absorbed light in the case of insufficient light; and in the case of excess of light protect their light harvesting machinery from the photo-oxidative damage.

Herbicides are often considered a 'two-edged sword' as they kill or slow the growth of unwanted herbs from crop fields on one hand while they may affect non-target crop plants and microorganisms on the other. Pretilachlor [2-chloro-2,6-diethyl-N-(2-propoxyethyl) acetanilide] is a selective pre- or post-emergence chloroacetanilide herbicide, widely used for the control of broad-leaved weeds, several grasses and sedges in transplanted and directly seeded paddy fields. According to the WSSA (Weed Science Society of America) pretilachlor has been categorised under group 15 and its mode of action is by inhibiting long chain fatty acids synthesis and cell division. The environmentally realistic doses of pretilachlor vary from 0.84 to 15.89 μg/L (i.e. 0.00084–0.01589 μg ml^{-1}) in Japanese rice fields (Phong et al., 2010) to 936–1233 μg L^{-1} (i.e. 0.936–1.233 μg ml^{-1}) in Italian paddy water (Vidotto et al., 2004). Many studies say that pretilachlor readily dissipates in rice fields by photodecomposition, microbial degradation and volatilization and the recommended rate do not affect soil properties or pose a serious problem for environment (Sahoo et al., 2016). However, Flori et al. (2003) found that more than half of the initially applied pretilachlor persisted in the surface water of laboratory microcosm after 10 days of treatment. The photolysis of pretilachlor follows the first order kinetics. Dissipation kinetics (DT50) of pretilaclor has been reported to be 3.0–3.6 days by Fajardo et al. (2000) while 8.8 days under reductive conditions and 17 d under oxidative conditions by Murata et al. (2004). The half-life of pretilachlor varied from 0.87 to 1.52 days for flood water (Dharumarajan et al., 2011). Paddy field cyanobacteria which have 4–10 days of life cycle may get affected by the repeated application of pretilachlor.

Generally, many environmental factors interact with each other in real field situation. Numerous studies have been done in the area of acclimatization/adaptation to various abiotic stresses with photosynthetic organisms (Miskiewicz et al., 2002). But, there are very few studies available about the altered behavior of differentially light acclimatized cyanobacterial cells (Deblois et al., 2013) when some sort of stress are being exerted at the same time (see the review of Gomes and Juneau, 2017). It will be extremely useful to know the impact of pretilachlor on some N_2-fixing cyanobacteria of paddy fields under different light acclimatizing conditions in the terms of their physiology, biochemistry and chlorophyll *a* fluorescence. So, the present study has been devoted to these objectives.

2. Materials and methods

2.1. Test organisms and culture conditions

Heterocystous cyanobacterial strains of *Anabaena* sp. PCC 7120 (hereafter *Anabaena* sp.) and *Nostoc muscorum* ATCC 27893 (hereafter *N. muscorum*) were cultivated in BG-11 medium (pH 7.5) and the homogenous cultures of both the cyanobacteria were maintained in a temperature controlled culture room at 25 ± 2 °C under 75 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR, 400–700 nm) with a 14:10 h of light:dark regime. Early exponential growth phase of both the cyanobacteria was used for the experiments.

2.2. Photoacclimatization and pretilachlor treatment

The early log phase cultures of *Anabaena* sp. and *N. muscorum* were acclimatized to the three different light intensities (suboptimum, 25 μmol photons m^{-2} s^{-1}; optimum, 75 μmol photons m^{-2} s^{-1}; and supra-optimum, 225 μmol photons m^{-2} s^{-1}) for 14:10 h light:dark photo-period adjusted by the number and position of the fluorescent tubes (Osram L 40W/25–1). This process of photoacclimatization was attained by regular sub-culturing in fresh nutrient medium for 24 days (6 generations, each comprising of 4 days). The detailed experiments were performed with differentially grown cyanobacteria under respective light intensities and the initial OD_{750} of each was adjusted at 0.1.

The effective doses of pretilachlor [trade name: Prince, EC: 50%, active ingredient: pretilachlor *i.e.* 2-chloro-2,6-diethyl-N-(2-propoxyethyl) acetanilide] were selected as 3 μg ml^{-1} and 6 μg ml^{-1} for the current study on the basis of several screening experiments and available literatures. Justifications behind the selection of these doses are that, these doses affected the growth of the cyanobacteria up to 30% and the effect of pretilachlor apprehended to be intensified or lessened under altered light regimes. Further, under laboratory conditions, up to 20 mg L^{-1} (i.e. 20 μg ml^{-1}) concentrations of pretilachlor have been used by Singh et al. (2016) for *Synechocystis* sp. while up to 40 mg L^{-1} (i.e. 40 μg ml^{-1}) concentrations of pretilachlor have been used by Inderjit and Kaushik (2010) in the case of *Anabaena fertilissima*. Concentrations of the range of 5–10 mg L^{-1} (i.e. 5–10 μg ml^{-1}) of pretilachlor could inhibit the nitrite and nitrate uptake by 35–37% in the cyanobacterium *Desmonostoc muscorum* as observed by Singh et al. (2015). In addition to these, the highest recommended dose of the pretilachlor as given by the producer (Krishi Rasayan Exports, Pvt. Ltd., Solan, Himachal Pradesh, India) is about 1500 μg ml^{-1}. These experiments satisfied the objective of studying the instantaneous effects of pretilachlor on the N_2-fixing cyanobacteria under laboratory conditions.

The photoacclimatized cyanobacterial cells were harvested by centrifuging them at 4000*g* for 10 min and washed twice with the distilled water and again a suspension of cyanobacterial pellets were prepared in a fresh nutrient medium containing two different (3 μg $ml^{-1}$and 6 μg ml^{-1}) doses of pretilachlor. After 72 h of pretilachlor treatment, different parameters were analyzed.

2.3. Measurement of growth

Growth was measured in the terms of dry mass. For this, a definite volume of treated and untreated cyanobacterial cells were harvested by centrifuging them at 4000*g* for 10 min and washed thrice with the distilled water. Cells were dried in an oven at 80 °C for 48 h and then weighed with a digital balance (Contech- CA 223, India).

2.4. Measurement of the photosynthetic pigments

For the extraction of chlorophyll *a* and carotenoids, 10 ml volume of each test sample was centrifuged and pellets were suspended in 2 ml