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Tolerance of *Anabaena* sp. PCC 7119 to cypermethrin measured through photosynthetic pigment fluorescence

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ABSTRACT

Insecticides are used worldwide in agriculture in vast amount every year. Use of pyrethroid insecticides has increased during past two decades due to their high knock down action and considerably low mammalian toxicity. Residual toxicity of the insecticides has also gone up due to their extensive use making it necessary to develop strategies for their rapid detoxification in the environment. The present study was undertaken to study the response of normal and cypermethrin pre-exposed *Anabaena* sp. PCC 7119 strains against the pyrethroid insecticide. Assay for 5 days showed enhanced growth performance of the tolerant strain up to 40 µM cypermethrin compared to that of normal strain. The stress indicating fluorescence parameters, viz., relative variable fluorescence at J level (V_j), net rate of PSII closure (M_0) and effective dissipation of active RC (DI_0/RC) were low in the strain having tolerance than of a normal one. Similarly, there was enhancement of the photosynthetic efficiency measured through the estimation of variable fluorescence (F_v) and fluorescence yield. The plant efficiency measured through derived fluorescence parameters, viz., maximum trapping rate of active PSII (TR_0/RC), trapping probability (TR_0/Abs), electron transport probability (ET_0/TR_0), and electron transport in active RC (ET_0/RC) showed higher values in the tolerant strain than in the normal one.

1. Introduction

Pyrethroids are widely used as pest control agents in a wide array of indoors and out door applications, including medicinal, veterinary and agriculture sector and are the synthetic derivative of natural pyrethrins with greater potenticy and environment stability (Elloiott and Janes, 1978). Cypermethrin was introduced on the market in late 1970s and has since been used on a wide range of crops due to its high pesticidal activity and low mammalian toxicity relative to other insecticides. Several recent studies show that pesticide residues frequently occur in surface water and soil in agricultural areas (Larson *et al.,* 1999). Many aquatic and soil algal and cyanobacterial species are known to be affected by the non-target effects of pesticides (Mohapatra and Mohanty, 1992; Mohapatra *et al.,* 1996, 2003; Barata *et al.,* 2006; Jena *et al.,* 2012). Aquatic ecosystems in agricultural fields are thus at risk of being negatively affected by these chemicals (Kallqvist and Ramstad, 1994; Ahmad, 2008; Cycon *et al.,* 2012; Tandon *et al.,* 2012; Jena *et al.,* 2012).

Insecticide resistance is an important feature under natural condition and many organisms ranging from the target insect(s) to bacteria are known to develop resistance on prolonged exposure to non-cidal concentrations of the insecticides (Kostaropoulos *et al.,* 2001; El-Latif and Subrahmaniyam, 2010). Pyrethroid resistance in insects has been recorded worldwide (Agosin, 1985; Wendt-Rasch *et al.,* 2003). Resistance has been found in bacteria though the exact mechanism of resistance is yet to be properly deciphered. Cytochrome P450 mediated detoxification of OP insecticides has been recorded in bacteria and cyanobacteria (Rodriguez *et al.,* 1993; Cheikh *et al.,* 1998). There are also informations on esterase mediated detoxification of OP chemicals and both general and specific esterases have

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been found effective to metabolically hydrolyse the insecticides (Sogorb *et al.,* 1998; Sogorb and Vilanova, 2002; Trovaslet-Leroy *et al.,* 2011). However, there is hardly any information of such activity against pyrethroid insecticides in cyanobacteria though the same has been recorded in many other organisms (Qu *et al.,* 2000; El-Latif and Subrahmaniyam, 2010; Tandon *et al.,* 2012). Moreover resistance of cyanobacteria against pyrethroid insecticides has not yet been documented. The purpose of the present study was to examine the effects of cypermethrin, a pyrethroid insecticide, on the normal and 30 µM cypermethrin tolerant *Anabaena* sp PCC 7119. The paper focuses on the effects of exposure to cypermethrin on the OIJP fluorescence transient and photosynthetic activity.

2. Materials and methods

The filamentous and heterocystous cyanobacterium *Anabaena* sp. PCC 7119 of normal and 30 µM cypermethrin tolerant strain was chosen as test organism for this work. The organism was maintained in non absorbent cotton stopper 250 ml flasks containing 250 ml B.G-11 medium (Rippka *et al.,* 1979). Actively growing cells were taken at an initial inoculums density with absorbance of 0.05 at 678 nm (3.664 ± 0.113 µg Chl *a* / 10^7 cells). The inocula were prepared in a sterile growth medium 2 h before the experiment. All stocks and experimental cultures were grown in a culture room at 29 ± 2^0C with a continuous irradiance of 35 µE/m^2 s. The stock solution (10 mM) of cypermethrin 25 EC [(R, S)-α – Cyano – 3 – phenoxybenzyl – 2, 2 – dimethyl (1R, 1S) cis, trans – (2, 2- dichlorovinyl) cyclopropane carboxylate] was freshly prepared by dissolving the commercial formulation of the insecticide in equal volume of acetone and diluted with freshly prepared aqueous medium. Required volumes of the stock solution were added aseptically to the experimental culture flasks/ tubes to achieve desired treatment concentrations (5-40 µM). The cultures were grown for 5 days under the growth conditions same as that of the stock cultures and then the effect was measured.

The absorbance of the homogenized culture suspension was measured at 678 nm and 630 nm, using UV -1700 pharmaspec UV - Vis spectrophotometer (Shimadzu, Japan), as growth parameters. The extraction of photosynthetic pigment was made in methanol following the standard extraction protocol for cyanobacteria and absorbance values of extracts were converted to biomass (mg pigment/ l of culture) following the equation of Hirschberg and Chamoviz (1994). Extraction and quantification of the cellular protein content was done by following the protocol of Lowry *et al.* (1951). The absorbance values were converted to biomass of protein using a standard curve prepared through same procedure taking bovine serum albumin as substrate. Measurement of carbohydrate content of cultures was made following the anthrone reagent method of Roe (1955). The absorbance values were converted to biomass of carbohydrate through the regression equation of a standard slope prepared by using glucose.

The chlorophyll *a* fluorescence was measured through the liquid culture attachment of a plant efficiency analyzer (Handy PEA, Hansatech Instruments, Norfolk, UK) following the protocol of Jena *et al.* (2012). The homogenized culture of the cyanobacterium was taken in 2 ml capacity glass vials fitted with aluminum screw cap and the vials were placed in dark for 15 minutes for complete relaxation of PS II RCs. Each vial was then thoroughly shaken and placed in the measuring chamber of the attachment. The measuring gain of the attachment and the PEA were fixed at 1.0 and 0.7 (rel units), respectively. The fluorescence was induced by a saturating white light pulse at an irradiance of 1500 µE/ m^2s provided from the internal light source of the liquid culture attachment. The fluorescence rise of O, J, I and P levels were recorded after 50 µs (F_0 µs), 2 ms (F_J), 30 ms (F_I) and T_{fm} (F_m), respectively. Several fluorescence parameters, viz., variable fluorescence (F_v), relative variable fluorescence at J level (V_j), net rate of PSII closure (M_0), maximum quantum yield of primary photochemistry (φP_0), probability of exciton movement beyond Q_A (Ψ_0), quantum yield of electron transport (φE_0), quantum yield of energy dissipation (φD_0), maximum trapping rate of active PSII (TR_0/RC), electron transport in active RC (ET_0/RC), effective dissipation of active RC (DI_0/RC), and the performance index of primary photochemistry (PI_φ) were calculated from the OJIP fluorescence transient following the equations of Force *et al*. (2003) and Strasser *et al.* (2005). The photosynthetic flux parameters, absorption flux, trapped energy flux, electron transport flux and dissipation flux per RC and the performance of primary photochemistry were also analyzed for estimation of the photosynthetic efficiency of cells.

The samples were taken in triplicate and treatments were made with graded concentrations of the insecticide. In all cases the stock culture used was the one, which was continuously maintained at 30 µM insecticide and other untreated normal strain. The data presented in tables and figures are the means of replicates of two successive experiments. Comparison among means have been done wherever necessary using standard statical methods (Gomez and Gomeez, 1984).

3. Results and discussion

Significant reduction in the culture absorbance at 678 nm and 630 nm on treatment of normal cultures with graded

concentrations of the insecticide. Concurrently there was similar pattern of the cellular carbohydrate and protein contents in the normal cultures (Table 1). On the other hand, the growth of the tolerant cultures were found significantly enhanced by the insecticide at concentrations ≤ 10 mM. The other higher concentrations ≤ 30 mM caused only insignificant reduction of growth and biochemical content of cells during the treatment period.

In normal strain of cyanobacterium a significant reduction of chl *a* and carotenoid content was observed with increase in the concentration of cypermethrin indicating down regulation of photosynthetic pigments for survival. The Chl *a*/ Car ratio did not differ significantly even though a slight increase in the ratio in the treated cultures was observed. This proved that both the photosynthetic pigments were more or less equally affected by the insectides. This is in agreement with the results observed on treatment with other insecticides (Mohapatra *et al.,* 1992; 1996; 2003; 2010; Jena *et al.,* 2012). A comparatively high amount of pigment content in the tolerant strains on treatment the same concentrations of the insecticide showed that the cyanobacterium was not adversely affected by the insecticide concentrations taken in the study though significant inhibition of growth and biochemical contents of cells were reported at these concentrations in the normal strain.

The cellular protein and carbohydrate content of normal strain decreased with increase in cypermethrin concentrations whereas the tolerant strain of cyanobacterium showed no significant change in the metabolite content (Table 1). This strengths the fact that the cyanobacterium was metabolically active in the presence of the insecticide concentrations, that are found growth inhibitory to the normal strain. Growth enhancement indicated that the cyanobacterium not only tolerated the insecticide in the applied concentration but also utilized the same as a nutrient source. Such phenomena have been reported in bacteria and cyanobacteria at low insecticide concentrations. It has also been reported that OP insecticides accelerate the growth of soil algae and cyanobacteria at field concentration (Mohapatra and Schiewer, 2000; Li *et al.,* 2010).

The variable fluorescence is an indicator of photosynthetic activity of green plants and the fluorescence responses are highly correlated with the chlorophyll content of the photosynthetic structure (Strasser *et al*., 1995; Elias *et al.,* 2004; Strasser *et al.,* 2005; Marques da Silva, 2007). Healthy plants show high variable fluorescence than of stressed ones (Strasser *et al.,* 1995; 2005; Marques da Silva, 2007). Cyanobacteria often show low variable fluorescence as the fluorescence rise in such organisms is significantly low compared to green plants (Mohapatra and Schiewer, 2000; Li *et al.,* 2010). It is because of the fact that a significant part of the antenna pigments in cyanobacteria are phycobiliproteins (organized as membrane complexes called phycobilisomes) which do not absorb the red light applied to generate fluorescence.

The shape of OJIP fluorescence rise has been found to be very sensitive to stress caused by changes in different environmental conditions, e.g., light intensity, temperature, drought, atmospheric CO_2 concentration and chemical influences (Tsimilli-Michael *et al.,* 2000; Strasser *et al.,* 2005; Antal and Rubin, 2008; Li *et al.,* 2010; Mohapatra *et al.,* 2010; Jena *et al.,* 2012). The observations showed that the shape of the OJIP rise and also that of the relative variable fluorescence [V(t)] was significantly changed only in normal strain (Fig.1) but such change was not reported in the tolerant strains in response to the treatment with the selected

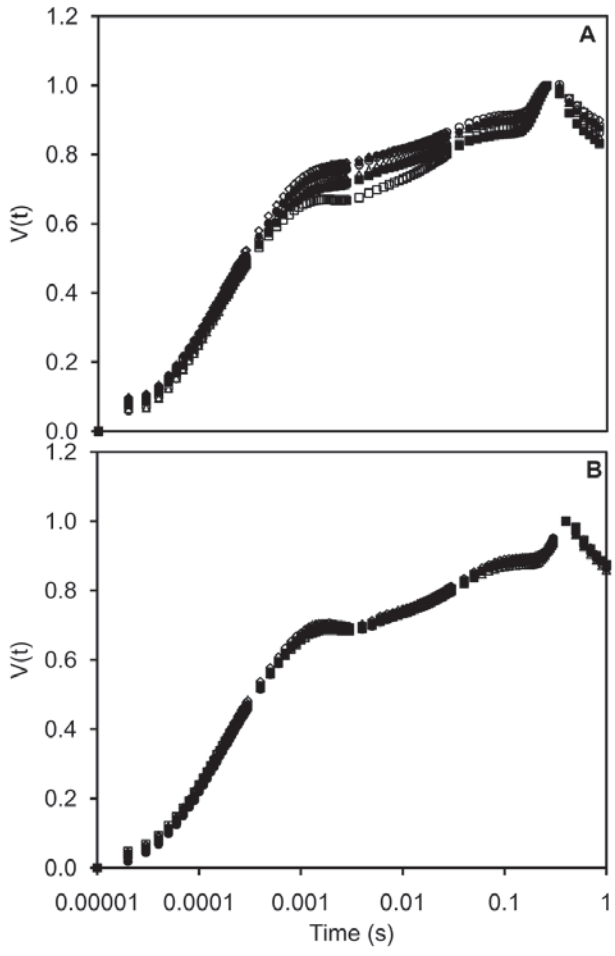

Fig.1. The OJIP fluorescence transient of normal and cypermethrin tolerant *Anabaena* sp. PCC 7119 strains exposed to different concentrations of the insecticide for 5 days. The transients represent the relative variable fluorescence [V(t)]. Cypermethrin concentrations (μM): (□)0, (■) 5, (△) 10, (o) 20, (●) 30, and (◇) 40.

Table 1
Effect of cypermethrin on growth and pigment content of normal and cypermethrin tolerant *Anabaena* sp. PCC 7119 strains during 5 days of incubation. C1 and C2 are the normal and tolerant control cultures, respectively.

Cypermethrin (μM)	678 nm	630 nm	678/630 nm	Chl *a* (mg/l)	Carotenoids (mg/l)	Chl *a*/Car	Carbohydrate (mg/l)	Protein (mg/l)
C1	0.742±0.007^{a}	0.736±0.004^{a}	1.008±0.044^{a}	6.019±0.085^{a}	1.602±0.044^{a}	3.76±0.116^{b}	104.2±3.4^{a}	332.1±12.8^{a}
5	0.631±0.004^{b}	0.654±0.004^{b}	0.965±0.038^{ab}	5.244±0.073^{b}	1.297±0.035^{b}	4.04±0.148^{a}	87.4±3.9^{b}	283.2±13.9^{b}
10	0.617±0.005^{c}	0.626±0.006^{c}	0.986±0.041^{a}	5.108±0.105^{c}	1.263±0.028^{c}	4.04±0.204^{a}	84.2±3.1^{b}	273.5±11.6^{c}
20	0.527±0.006^{d}	0.579±0.007^{d}	0.911±0.028^{ab}	4.352±0.093^{d}	1.092±0.031^{d}	3.98±0.182^{a}	71.1±4.0^{c}	231.5±14.2^{d}
30	0.461±0.002^{e}	0.508±0.007^{e}	0.906±0.046^{b}	3.337±0.082^{e}	0.873±0.037^{e}	3.83±0.179^{ab}	51.5±3.2^{d}	172.4±5.8^{e}
40	0.372±0.019^{f}	0.473±0.005^{f}	0.786±0.029^{c}	2.825±0.066^{f}	0.704±0.034^{f}	4.01±0.227^{a}	39.7±1.4^{e}	132.6±5.7^{f}
C2	0.743±0.005^{b}	0.747±0.006^{b}	0.995±0.043^{a}	5.987±0.142^{b}	1.611±0.069^{b}	3.72±0.213^{b}	101.3±4.1^{b}	328.1±12.9^{c}
5	0.880±0.006^{a}	0.870±0.006^{a}	1.011±0.051^{a}	7.127±0.133^{a}	1.859±0.073^{a}	3.83±0.128^{ab}	123.1±3.9^{a}	397.4±17.3^{a}
10	0.874±0.003^{a}	0.871±0.006^{a}	1.003±0.032^{a}	6.921±0.097^{a}	1.769±0.022^{ab}	3.91±0.187^{ab}	117.4±4.2^{a}	376.1±18.3^{b}
20	0.739±0.005^{b}	0.725±0.007^{c}	1.019±0.037^{a}	5.922±0.109^{b}	1.588±0.038^{c}	3.72±0.117^{b}	104.4±5.2^{b}	388.7±11.2^{ab}
30	0.735±0.005^{b}	0.718±0.006^{cd}	1.023±0.042^{a}	5.864±0.117^{b}	1.579±0.059^{c}	3.71±0.132^{b}	101.2±5.6^{b}	332.4±14.7^{c}
40	0.704±0.001^{c}	0.702±0.002^{d}	1.002±0.029^{a}	5.589±0.138^{c}	1.388±0.042^{c}	4.02±0.185^{a}	94.2±3.2^{c}	286.4±11.2^{d}

Note: Means superscripted by same letter in a column are not significantly different from each other at p = 0.05; comparison was done separately for normal and tolerant strain by applying LSD.

Table 2
The OJIP fluorescence derived parameters of normal and cypermethrin tolerant *Anabaena* sp. PCC 7119 strains exposed to different concentration of the insecticide for 5 days. C1 and C2 are as mentioned in table 1.

Cypermethrin (μm)	Fv	V_j	M_0	φP_0	ψ_0	φE_0	φD_0	TR_0/RC	ET_0/RC	DI_0/RC	PI_φ
C 1	478[a]	0.582[d]	1.425[c]	0.281[a]	0.418[a]	0.117[a]	0.719[e]	2.448[b]	1.023[a]	6.265[f]	0.391[a]
5	428[b]	0.615[c]	1.576[c]	0.232[b]	0.385[b]	0.089[b]	0.768[d]	2.563[a]	0.987[b]	8.483[e]	0.302[b]
10	359[c]	0.674[b]	1.661[b]	0.214[c]	0.326[c]	0.070[c]	0.786[cd]	2.464[b]	0.803[c]	9.051[d]	0.272[c]
20	320[d]	0.709[a]	1.725[b]	0.201[d]	0.291[d]	0.058[d]	0.799[bc]	2.433[b]	0.708[d]	9.671[c]	0.252[d]
30	277[e]	0.718[a]	1.811[a]	0.183[e]	0.282[e]	0.052[de]	0.817[ab]	2.522[ab]	0.711[d]	11.261[b]	0.224[e]
40	203[f]	0.721[a]	1.859[a]	0.168[f]	0.279[e]	0.047[e]	0.832[a]	2.578[a]	0.719[d]	12.769[a]	0.202[f]
C 2	467[a]	0.609[bc]	1.467[b]	0.287[a]	0.391[a]	0.112[a]	0.713[b]	2.409[a]	0.942[a]	5.984[bc]	0.403[a]
5	464[a]	0.634[b]	1.498[b]	0.277[a]	0.366[bc]	0.101[b]	0.723[b]	2.363[a]	0.865[b]	6.167[b]	0.383[a]
10	455[a]	0.645[b]	1.392[b]	0.278[a]	0.355[cd]	0.099[b]	0.722[b]	2.158[b]	0.766[cd]	5.605[cd]	0.385[a]
20	478[a]	0.652[b]	1.298[c]	0.286[a]	0.348[d]	0.100[b]	0.714[b]	1.991[c]	0.693[d]	4.970[d]	0.401[a]
30	460[a]	0.625[bc]	1.372[bc]	0.283[a]	0.375[b]	0.106[ab]	0.717[b]	2.195[b]	0.823[bc]	5.562[cd]	0.395[a]
40	412[b]	0.694[a]	1.689[a]	0.238[b]	0.306[e]	0.073[c]	0.762[a]	2.434[a]	0.745[cd]	7.792[a]	0.312[b]

Note: Means superscripted by same letter in a column are not significantly different from each other at p = 0.05; comparison was done separately for normal and tolerant strain by applying LSD.

concentrations of the insecticide. There was a rise in the fluorescence at J level in the normal strain and this increased with the increase in the insecticide concentration. Variable fluorescence and fluorescence yield (φP_0) showed a higher value in tolerance strain then that of normal one. Treatment of the cyanobacterium with 40 μM of cypermethrin caused high values of the V_J, M_0, φD_0 and DI_0/RC whereas the low values of performance parameters ψ_0, φE_0, TR_0/RC and ET_0/RC were reported indicating that at this concentration of the insecticide the cyanobacterium was at stress in both normal and tolerant strains (Table 2). No significant variation in these parameters were observed at concentrations ≤ 30 mM of cypermethrin in the tolerant strain indicating that the cyanobacterium could become able to tolerate these concentrations of the insecticide but not so in the normal strain.

Lazar (2003) has observed that J fluorescence rise (the slope of initial fluorescence rise) is caused by an increase in the accumulation of excited states that are formed when Q_A is reduced and when Q_A is reduced together with single- or double- reduced Q_B. There is also a relative increase of F_0 with 40 μM of the insecticide, indicating the increase in Q_A reduction at the beginning but improvement of multiple turn over events with adaptation (Mohapatra *et al.,* 2010; Li *et al.,* 2010). In tolerance strain there is a stabilization in the PSII-PSI electron flow indicating its well adaptation to high concentrations of insecticide compared to the normal strain.

4. Conclusion

The study showed that prolonged exposure of *Anabaena* sp. PCC 7119 to growth inhibitory concentrations of cypermethrin induced tolerance of the cyanobacterium to the insecticide. The growth and photosynthetic efficiency of the cyanobacterium was not significantly affected by the insecticide at concentrations ≤ 30 mM whereas the insecticide could strongly inhibited the growth and metabolic performance of normal strain of the cyanobacterium. The tolerant strain of *Anabaena* sp. PCC 7119 could grow well at concentrations much higher than that possible in the environment achieved after commercial application of the insecticide. Thus it can be inferred that the cyanobacterium can be a good candidate for accelerated degradation of the insecticide of contaminated fields.

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