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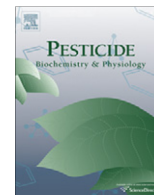

Effects of insecticide acetamiprid on photosystem II (PSII) activity of *Synechocystis* sp. (FACHB-898)

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ABSTRACT

Effects of insecticide acetamiprid on photosystem II (PSII) activity of *Synechocystis* sp. were investigated by a variety of in vivo chlorophyll fluorescence tests. Acetamiprid exposure increased the proportion of inactivated PSII reactive centers ($PSII_X$) and led to loss of active centers ($PSII_A$). High concentration (1.0 mM) acetamiprid decreased amplitude of the fast phase and increased the slow phase of fluorescence decay during Q_A^- reoxidation. The electron transport after Q_A was hindered by high concentration acetamiprid and more Q_A had to be reoxidized through $S_2(Q_AQ_B)^-$ charge recombination. Acetamiprid decreased the density of the active reaction centers, electron transport flux per cross section and the performance of PSII activity but had little effect on dissipated energy flux per reaction center, antenna size and the maximum quantum yield for primary photochemistry (F_v/F_m). The target site of acetamiprid toxicity to the PSII of *Synechocystis* sp. was electron transfer on the acceptor side.

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1. Introduction

Acetamiprid is a systemic and contact insecticide belonging to neonicotinoids group. Acetamiprid acts as an agonist of acetylcholine by binding to nicotinergic acetylcholine receptors (*n*AChR) on the post-synaptic membrane [1,2]. Owing to its broad insecticidal spectrum and relatively low acute and chronic mammalian toxicity, acetamiprid is used widely in crop protection [3,4]. Though the half life of aetamiprid in field was reported to be about 2.8–14 days [5], the risk of its ambient pollution, principally in water, is still present [2]. Acetamiprid exposure has been proven to have adverse effect on greenhouse workers spraying acetamiprid [6], soil microorganisms [4] and beneficial insects [3]. However, effects of acetamiprid on the photosynthetic apparatus of aquatic microorganisms such as cyanobacteria are little known.

Cyanobacteria occupy the lower trophic levels within food webs. Changes in their community may have indirect but significant effects on the rest of the freshwater communities. Therefore, toxicological effects of hazardous chemicals on cyanobacteria are frequently studied. Photosynthesis is the principal mode of energy metabolism in cyanobacteria. Photosystem II (PSII) is thought to be the primary and sensitive site of inhibition induced by a wide range of environmental pollutants [7–9]. Recent studies showed that PSII activities of cyanobacteria were significantly inhibited by some organic pollutants [10,11] and heavy metals [12,13]. In the case of insecticides, it was found that dimethoate caused inhibition of photosynthetic electron transport and photosynthetic carbon fixation but increase of PS II fluorescence of *Synechocystis* sp. PCC 6803 [9].

This study aimed at investigating the effects of insecticide acetamiprid on electron transport, energy flux and heterogeneity of reaction centers in PSII of *Synechocystis* sp. by in vivo chlorophyll *a* fluorescence tests.

2. Materials and methods

2.1. Culture of cyanobacterium

Synechocystis sp. (FACHB-898) cells were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The test organism was grown in BG-11 medium [14] at 30 °C under continuous fluorescent white light (55 μmol photons m^{-2} s^{-1}) with a 16:8 h light–dark cycle. The cultures were agitated with hands every 6 h. The growth of cultures was monitored every 12 h by measuring cell optical density at 625 nm (OD_{625}) with a spectrophotometer (UV-2800, Unico, Shanghai, China). The growth phases of algological cultures were determined using a graphical method. The cells were harvested in exponential growth phase and then transferred to 10 mm × 10 mm plastic cuvettes filled with acetamiprid bearing BG-11 medium at 15 μg chlorophyll per milliliter.

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2.2. Acetamiprid treatment

The acetamiprid (97% of purity) was purchased from Dongfeng Insecticide Factory, Shanghai, China. The suspension in each cuvette was diluted to the same Chl density by addition of acetamiprid solution and/or BG-11 medium, and the final acetamiprid concentration ranged from 0.05 to 1.0 mM. The higher concentrations than observed in the environment were also encompassed in order to elicit measurable toxic responses. The sample with 0 mg L^{-1} acetamiprid was used as the control. All the samples untreated and treated with acetamiprid were kept in suspension by stirring and incubated at 25 °C under continuous fluorescent white light (55 μmol photons m^{-2} s^{-1}).

2.3. Chl a fluorescence measurement

A double-modulation fluorometer (FL3500, PSI, Inc., Brno, Czech) was employed to measure the polyphasic fast fluorescence induction, Q_A^- reoxidation kinetics, and the proportion of active and inactive reaction centers. The sample concentration was about 15 μg chlorophyll per milliliter. All the samples were dark-adapted for 1 min before each test.

2.3.1. Polyphasic fast fluorescence induction and JIP-test

The chlorophyll fluorescence transients were recorded up to 1 s on a logarithmic time scale, with data acquisition every 10 μs for the first 2 ms and every 1 ms thereafter. The saturating flash intensity was set as 60% of the power. Each measured O–J–I–P induction curve was analyzed according to the JIP-test [15,16]. The following data were directly obtained from the fast rise kinetic curves: F_o, the initial fluorescence, was measured at 50 μs, at this time all reaction centers (RCs) were open; F_J and F_I are the fluorescence intensity at J step (at 2 ms) and I step (at 30 ms); F_m, the maximum fluorescence, was the peak fluorescence at P step when all RCs were closed after illumination; $F_{300\ \mu s}$ was the fluorescence at 300 μs. The following selected JIP-test parameters qualifying PSII behavior were calculated from the above original data [15]: V_J, relative variable fluorescence at the J-step; M_O, approximated initial slope of the fluorescence transient; ABS/CS_O, absorption flux per CS_O, approximated by F_O; TR_O/CS_O, trapped energy flux per CS_O; ET_O/CS_O, electron transport flux per CS_O; DI_O/CS_O, dissipated energy flux per CS_O; RC/CS_O, density of RCs Q_A^- reducing PSII reaction centers); Ψ_O, probability (at $t = 0$) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; $\Psi P_O = F_v/F_m$, maximum quantum yield of primary photochemistry (at $t = 0$); φ_{EO}, quantum yield of electron transport (at $t = 0$); φ_{DO}, quantum yield (at $t = 0$) of energy dissipation; PI_{ABS}, performance index on absorption basis; PI_{CS}, performance index on cross section basis.

2.3.2. Measurement of Q_A-reoxidation kinetics

The measurement of Q_A-reoxidation kinetics was performed by a single turnover flash. In this study, the Q_A^- reoxidation kinetics curves after a single turnover flash were measured in the 200 μs to 60 s range. Both actinic (30 μs) flashes and measuring (2.5 μs) flashes were provided by red LEDs. The measuring flash intensity was set as 100% of the power. The Q_A^- reoxidation kinetics data were recorded with eight data point per decade.

The Q_A^- reoxidation kinetics curves were fitted by the three-component exponential Eq. (1):

$$F(t) - F_0 = A_1 \exp(-t/T_1) + A - 2\exp(-t/T_2) + A_3 \times \exp(-t/T_3) \quad (1)$$

where $F(t)$ is the variable fluorescence yield at time t; F_0 is the fluorescence level before the flash; A_1–A_3 are the amplitudes; T_1–T_3 are the time constants. The nonlinear correlation between the fluorescence yield and the redox state of Q_A was corrected for using the Joliot model [17] with a value of 0.5 for the energy-transfer parameter between PSII units.

2.3.3. S-state test of inactive PSII ($PSII_X$) centers

The fluorescence decay is controlled largely by the reoxidation kinetics of Q_A^-. In active PSII ($PSII_A$) centers the oxidation of Q_A^- is rapid, whereas in inactive centers ($PSII_X$) the oxidation of Q_A^- is much slower [18]. The number of inactive RCs in the whole cells can be measured by the S-state test. The measuring flash intensity was set as 50% of the power. The F_o fluorescence is measured during the first 1 ms. 10 actinic flashes are fired 110 ms apart to advance the S-states. After each actinic flash, the fluorescence decay is measured. The population of $PSII_X$ centers was estimated by the different between the fluorescence level 110 ms after the fourth flash and F_0 ($\Delta F_{440\ ms}/F_0$-1) [11] because the fluorescence decay after the fourth flash is controlled almost entirely by inactive centers [19].

3. Results

3.1. Effect of acetamiprid on the fast fluorescence rise

The fast fluorescence induction kinetics of the control and 24-h acetamiprid treated samples was shown in Fig. 1. Effect of acetamiprid on the fast rise fluorescence of *Synechocystis* sp. was clearly concentration-dependent. The fluorescence intensities of the OJIP curve decreased almost in parallel with increasing acetamiprid concentration. It was also observed that fluorescence rise slowly from J step to I step for the control and low concentration acetamiprid stressed cells. At 1.0 mM of acetamiprid, the J–I phase rise almost disappeared.

More photochemical information was obtained using JIP-test analysis of the OJIP curves, and the selected JIP-test parameters were summarized in Table 1. It was found that the maximum quantum yield for primary photochemistry (F_v/F_m) of *Synechocystis* sp. was not significantly affected after exposure to acetamiprid. M_O decreased by about 4% after 24-h treatment with 0.05 and 0.1 mM acetamiprid. However, after exposure to 1.0 mM acetamiprid, M_O changed little, with respect to the control. The values of ABS/RC, TR_0/RC and quantum yield of energy dissipation (φ_{D0}) changed little under stress of various concentrations of acetamiprid, indicating that acetamiprid had little effect on energy flux per reaction center (RC). Electron transport (φ_{E0}), probability that a trapped exciton moves an electron into the electron transport chain beyond

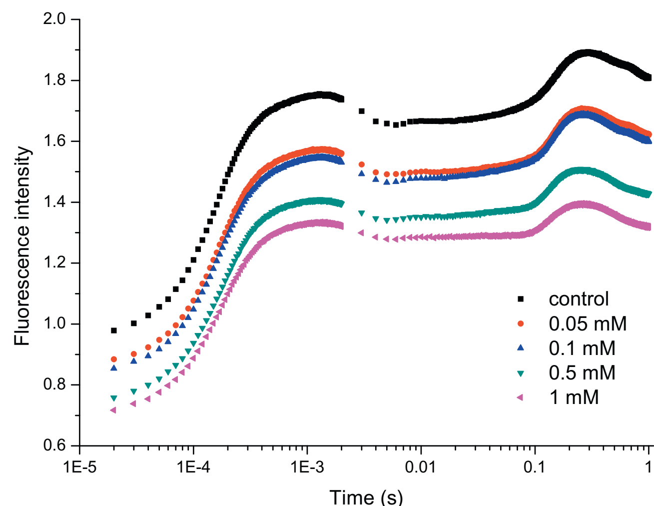

Fig. 1. The fluorescence transient of *Synechocystis* sp. untreated and treated for 24 h with various concentrations of acetamiprid. Each value was mean of 4 replicates.

Table 1
The JIP-test parameters of *Synechocystis* sp. cells cultured in various concentrations of acetamiprid for 24 h. All parameters were normalized to the control.

	V_J	M_0	F_v/F_m	ψ_o	φ_{Eo}	φ_{Do}	ABS/RC	TRo/RC	ETo/RC	RC/CSo	ETo/CS	PIcs	PIabs
Control	1	1	1	1	1	1	1	1	1	1	1	1	1
0.05 mM	0.992	0.966	0.998	1.036	1.034	1.002	0.976	0.974	1.009	0.925	0.935	0.962	1.064
0.1 mM	0.978	0.962	1.024	1.104	1.129	0.977	0.961	0.984	1.087	0.907	0.987	1.069	1.222
0.5 mM	1.022	0.994	1.03	0.894	0.92	0.972	0.944	0.972	0.87	0.821	0.715	0.762	0.978
1.0 mM	1.074	1.038	1.007	0.652	0.657	0.993	0.959	0.967	0.631	0.763	0.481	0.469	0.639

$Q_A^-(\Psi_0)$ (Ψ_0) and performance index (PI_{CS} and PI_{ABS}) were promoted by lower concentration (0.1 mM and below) acetamiprid but remarkably reduced by higher concentration acetamiprid (0.5 mM and higher). However, on the basis of cross section, the active photosynthetic reaction centers (RC/CS_0), absorption flux (ABS/CS), trapped energy flux (TR_0/CS) and electron transport flux per excited cross section (ET_0/CS) clearly decreased with increasing acetamiprid concentration.

3.2. Effect of acetamiprid on Q_A^- reoxidation kinetics

The Q_A^- reoxidation kinetics curves of *Synechocystis* sp. treated with various concentrations of acetamiprid were shown in Fig. 2. The amplitude of the variable fluorescence (F_v) decreased with increasing acetamiprid concentration and the decay rate was slowed down. The Q_A^- reoxidation kinetics parameters derived from the curves were summarized in Table 2. Generally, the fast phase, being 84.1–88.2%, dominated the reoxidation kinetics for both the control and acetamiprid treated samples. Q_A^- reoxidation kinetics was not significantly affected by lower concentrations (0.1 mM or below) of acetamiprid. A slight rise in amplitude of the fast phase and a slight decline in the slow phrase were observed at 0.1 mM acetamiprid with respect to the control. As acetamiprid concentration exceeded 0.5 mM, amplitude of the fast phase decreased significantly, accompanied with the increase of amplitude of the slow phase. For example, in the presence of 1 mM acetamiprid, the fast phase decreased by 4.1% while the slow phase increased by 3.14%. The amplitude of the middle phase changed slightly under stress of various concentrations of acetamiprid.

3.3. Effect of acetamiprid on inactive PSII ($PSII_X$) centers

The S-state test curves for the control and acetamiprid treated samples were shown in Fig. 3. Number of inactive PSII ($PSII_X$) centers increased linearly with increasing acetamiprid concentration (Fig. 4). The proportion of $PSII_X$ centers increased from 2.01% for the control to 13.9% for the sample treated with 1.0 mM acetamiprid.

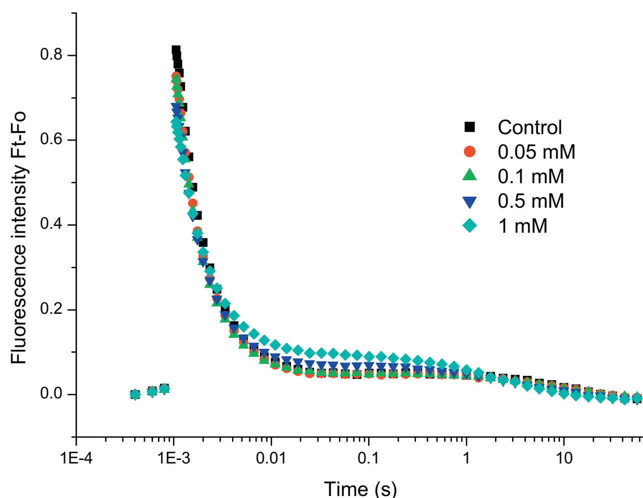

Fig. 2. The Q_A-reoxidation kinetics of *Synechocystis* sp. cultured for 24 h in BG-11 medium containing various concentration acetamiprid. Each value represented the mean of four replicates.

4. Discussion

In the present study, we have demonstrated that high concentration (0.5 mM or higher) acetamiprid had adverse effects on the PSII activity of *Synechocystis* sp. Acetamiprid decreased the density of active photosynthetic reaction centers per excited cross and quantum yield of electron transport, resulting in the decline of performance of PSII.

The Q_A^- reoxidation kinetics can be used for testing the effect of environmental stresses on the function of the acceptor side of PSII [20,21]. A single turnover saturating flash to dark adapted samples produces a high fluorescence yield. The subsequent fluorescence decay in the dark exhibits three main decay phases. The fast phase indicates electron transfer from Q_A^- to Q_B^- site occupied with Q_B Q_B^- before the actinic flash. The middle phase is typical for PSII complexes where Q_A^- reoxidation is limited by diffusion of PQ molecules to an empty Q_B-site [20]. The slower phase represents the charge recombination from the $S_2Q_A^-$ state of water oxidation to the S_1Q_A [22]. In the present study, the fast phase is the overwhelming majority for both the control and samples treated with various concentrations of acetamiprid, indicating that the Q_A^- reoxidation was mainly fulfilled by the electron transfer from Q_A^- to QB/Q_B^-. In the presence of acetamiprid at 0.1 mM or below, the amplitude of the fast phase and the middle phase rise slightly, accompanied with a slight drop of the amplitude of the slow phase. This means that low concentrations of acetamiprid may stimulate electron transport from Q_A^- to QB/Q_B^-. High concentration (1.0 mM) acetamiprid induced a decrease of the amplitude of the fast phase, an increase of amplitude of the slow phase and a slight increase of amplitude of the middle phase, implying that the contribution of $S_2(Q_AQ_B)^-$ charge recombination to Q_A^- reoxidation increased while electron transfer from Q_A^- to Q_A^- was hindered. More Q_A^- were forced to be oxidized with $S_2(Q_AQ_B)^-$ charge recombination [11].

The slow rise of J–I phase for the control and low concentration acetamiprid stressed cells suggests that reduced Q_A^- accumulated by slowing of its reoxidation because of reduction of Q_B and the quinone pool. The disappearance of this J–I phase rise at 1.0 mM acetamiprid implies that the reduction of the secondary quinone electron acceptor-Q_B, plastoquinone-PQ, cytochrome-Cyt and plastocyanin-PC may be inhibited by high concentration acetamiprid [23]. Besides, the effect on J–I phase might also be related to the membrane potential changes may also affect the J–I phase [24].

The JIP-test analysis of the chlorophyll fluorescence transient provides important information on the absorption, distribution and utilization of energy [15]. The JIP-test analysis shows that acetamiprid inhibits electron transport on the acceptor side of PSII of *Synechocystis* sp. Acetamiprid decreased the density of active reaction centers per cross section (RC/CS), resulting in the decrease of absorption flux, trapped energy flux and electron transport flux per excited cross section (ABS/CS, TR_0/CS, ET_0/CS). This was in accordance with the increase of the number of $PSII_X$ center. The

Table 2
Kinetic deconvolution of fluorescence decay kinetics of *Synechocystis* samples untreated and Acetamiprid treated. A_1, A_2 and A_3 are the amplitudes. T_1, T_2 and T_3 are the time constants. Data represented mean ± SE of four replicates.

Treatment	Fast phase		Middle phase		Slow phase	
	A_1 (%)	T_1 (μs)	A_2 (%)	T_2 (ms)	A_3 (%)	T_3 (S)
Control	88.2 ± 0.5[ab]	611.2 ± 14[a]	9.95 ± 0.4[a]	3.86 ± 0.08[a]	1.83 ± 0.12[a]	12.1 ± 0.4[a]
0.05 mM	88.6 ± 0.5[ab]	587.9 ± 16[ab]	9.70 ± 0.4[a]	3.70 ± 0.11[a]	1.71 ± 0.11[a]	10.1 ± 1.1[ab]
0.1 mM	89.6 ± 0.5[a]	561.1 ± 19[b]	8.79 ± 0.4[ab]	3.52 ± 0.20[a]	1.65 ± 0.16[a]	8.06 ± 0.7[b]
0.5 mM	87.5 ± 0.7[b]	615.6 ± 13[a]	9.67 ± 0.4[a]	3.94 ± 0.10[a]	2.82 ± 0.31[b]	4.28 ± 0.5[c]
1.0 mM	84.1 ± 0.8[c]	701.1 ± 8[c]	10.9 ± 0.5[b]	4.60 ± 0.14[b]	4.97 ± 0.40[c]	2.66 ± 0.2[c]

[abc] Data were analyzed by ANOVA followed by comparison between groups using the Student–Newman-Keuls test. Different letters of superscript denoted significant differences at $p = 0.005$. In the same columns with the same letters denoted no significant difference at $p = 0.005$.

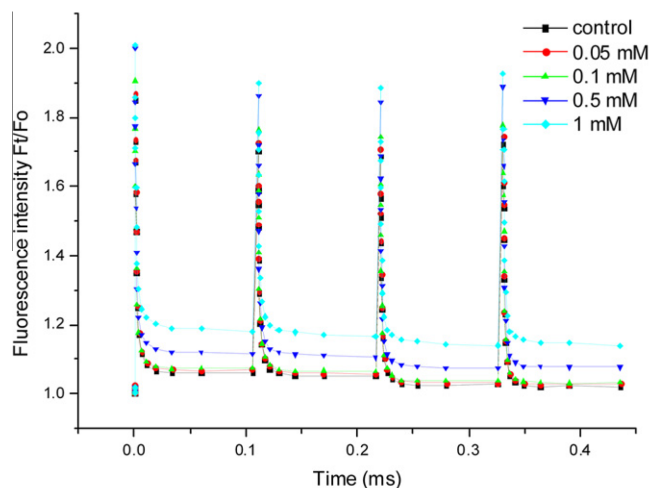

Fig. 3. The fluorescence decay induced by a series of single-turnover flashes for the control and samples treated for 24 h with various concentrations of acetamiprid. Each value represented mean of four replicates.

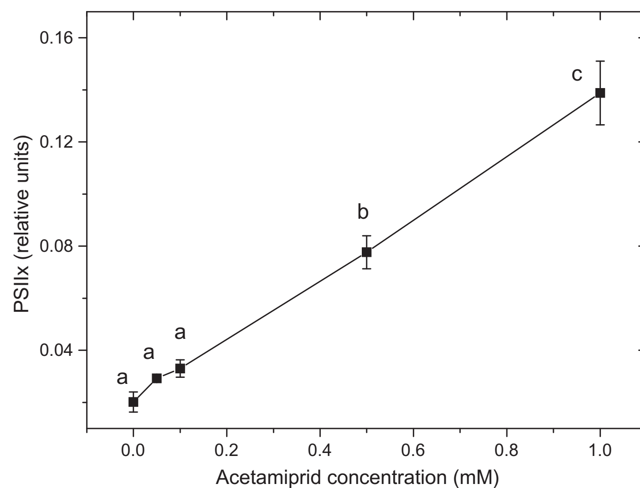

Fig. 4. Proportion of $PSII_X$ of *Synechocystis* sp. untreated and treated with various concentrations of acetamiprid for 24 h. Each value represented mean of four replicates and bars indicated the standard errors. [abc]Data were analyzed by ANOVA followed by comparison between groups using the Student–Newman-Keuls test. Different letters of superscript denoted significant differences at $p = 0.005$. In the same columns with the same letters denoted no significant difference at $p = 0.005$.

decrease of the number of the active centers caused the decrease of quantum yield for electron transport (φ_{E0}), probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (Ψ_0), and finally resulted in substantial decreases of performance indexes (PI_{CS} and PI_{ABS}). Heavy metals [12,13], antibiotics [11,25] and herbicides [26] have also been reported to inactivate the reaction centers and reduce the performance index. It is necessary to noted that in these previous studies the decrease of performance index was ascribed to a decrease of the density of the active reaction centers and an increase of dissipated energy flux and antenna size. However, in the present study, neither the antenna chlorophylls per reaction center nor dissipated energy flux (φ_{D0}) is significantly affected by acetamiprid treatment. The decrease of performance index is due to the decreases of energy flux and electron transport per cross section. This indicates that the decrease of quantum yield (φ_{E0}) induced by high concentration acetamiprid is only associated with inhibition of electron transfer from Q_A^- to Q_B to PQ, which is confirmed by evidence from the Q_A-reoxidation kinetics. In addition, M_O was not significantly affected by acetamiprid. M_O reflects the initial slope of the O–J growth phase on the fluorescence time course. This implies that acetamiprid did not significantly affect light induced single reduction of Q_A, i.e., net rate of PSII closure [7,28].

Several insecticides such as dimethoate [7,9] and lindane [29] were reported to significantly decrease the maximum quantum yield of primary photochemistry (F_0/F_m). However, in the present study, acetamiprid did not have significant effect on F_v/F_m while the value of performance index (PI_{ABS} or PI_{CS}) increased after exposure to low concentrations of acetamiprid. Only high concentration (1.0 mM) acetamiprid treatment resulted in a remarkable decrease of performance index. F_v/F_m has been frequently used as one sensitive index for PSII activity of photosynthetic samples treated with freezing [30], heat [31] and pollutants [32]. However, F_v/F_m represented only amount of energy trapped in PSII RCs in relation to energy absorbed [33] and damage to photosynthetic apparatus triggered by pool size of acceptors may not change F_v/F_m [34]. Unlike F_v/F_m, PI_{ABS} or PI_{CS} integrates much more information on PSII function including antenna, reaction center and energy fluctuations [33]. In the present study, acetamiprid reduced the pool size and inhibited electron transport on the acceptor side but had little effect on energy absorption and energy trapped. Therefore, F_v/F_m does not change significantly in response to acetamiprid while performance indexes respond sensitively to acetamiprid treatment.

In summary, our study showed that high concentration acetamiprid reduces photosynthetic performance of *Synechocystis* sp. by inhibiting its electron transport per cross section and inactivating RCs. However, acetamiprid exposure had little effect on antenna size, energy trap and dissipation of active RCs.

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