

RESEARCH ARTICLE

Determination of changes in *Arthrospira platensis* antioxidant activity and growth parameters due to oxidative stress arising from Lambda cyhalothrin

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Abstract – Toxic stress caused by pesticides changes the function and structure of the aquatic ecosystem via impressing to species composition. Therefore it is necessary to determine the reaction of cyanobacteria to pesticides for comprehend the effects of these substances on the aquatic ecosystems. This study aims to determine the toxicity and oxidative stress that Lambda cyhalothrin may cause in cyanobacteria, one of the primary producers in lake ecosystems. For these reasons, the changes in chlorophyll-*a* content, OD560 absorbance, the antioxidant enzyme activities such as superoxidodismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) were assessed to carry out the effect of Lambda cyhalothrin concentrations (between 6.25 and 100 $\mu\text{g mL}^{-1}$) on *Arthrospira platensis*. EC50 value is calculated as 11.94 $\mu\text{g mL}^{-1}$ Lambda cyhalothrin concentrations. SOD and APX activities was statistically different from the control at 100 $\mu\text{g mL}^{-1}$ Lambda cyhalothrin application compared to control in *A. platensis*-M2 cells. On the other hand, GR activity did not effect significantly. According to our results, we may conclude that Lambda cyhalothrin concentrations used in this study inhibited the growth of *A. platensis* cells in a time and dose-dependent manner, as indicated by lowered chlorophyll-*a* content and OD560 values and Lambda cyhalothrin caused oxidative stress in *A. platensis* cells. As a result, the restriction of Lambda cyhalothrin using at the certain concentrations may be a step to prevent pesticide pollution in the environment.

Keywords: Aquatic toxicology / cyanobacteria / SOD / GR / APx

1 Introduction

In recent years various chemicals included the pesticides have been released to the environment via modern industrial and agricultural activities (WHO, 1984; Asal, 1985; FAO/WHO, 1991; Bai and Ogbourne, 2016). Although the pesticides have enhancing effects on product quality and quantity, it is obvious that the irregular and uncontrolled using causes great damage in the ecosystem (Dere and Sivaci, 2003). Pesticides are important pollutants in the aquatic environment as a result of industrial and agricultural applications and they constitute a threat to living organisms (WHO, 1984; Asal, 1985; FAO/WHO, 1991). Pyrethroid compounds is a class of pesticides obtained from chrysanthemum flowers (*Chrysanthemum cinerariaefolium*), and they are frequently used in agriculture, public health, homes and gardens (Amweg and Weston, 2005; Oros and Werner, 2005).

During the use in agricultural and industrial areas, this compounds cause contamination by mixing the water with irrigation water, rainwater, and wastewater (Rondon and Caguan, 1994). Being transported to the aquatic ecosystem in various ways, these chemicals affect adversely to flora and fauna (Ernst *et al.*, 2001; Pereira *et al.*, 2009; Sanchez-Bayo, 2012).

Lambda-cyhalothrin is a pyrethroid compound (Robson and Crosby, 1984). The solubility is 5 $\mu\text{g L}^{-1}$ and its half-life is 21.9 days in water (He *et al.*, 2008). It is stable against heat and light. Hydrolysis may occur in alkaline mediums. Being lipophilic compounds can be absorbed through the membranes in the organism and it displays some features as accumulation in the tissue (Anadon *et al.*, 1996; Kidd and James, 1991; Karadag and Kaplan, 2016). Lambda-cyhalothrin is highly toxic and LC50 (96h) value is very low for aquatic organisms (0.8 ng L^{-1} for sheepshead minnow, 4.9 ng L^{-1} for mysid shrimp, 210 ng L^{-1} for bluegill sunfish, 240 ng L^{-1} for rainbow trout, and 360 ng L^{-1} for *Daphnia magna*) (He *et al.*, 2008).

Cyanobacteria, the primary producers, have an important role in the aquatic ecosystem due to being the main constituent

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of the food chain. The toxic effects of pesticides which are caused by not being degradation for a long time have important effects on these organisms and zooplankton due to feeding with phytoplankton. Thus, it can be concluded that the aquatic ecosystem is exposed to prolonged pesticide effects and that the primary productivity of water can be virtually destroyed (Burkiewicz *et al.*, 2005; Öterler and Albay, 2016). *A. platensis* is a spiral-shaped prokaryotic organism, usually consisting of cylindrical cells which are a few millimeters in length and their diameter are variable between 3 and 12 μm . This organism can grow at high temperatures (35–38 °C) and in a high alkaline environment. *A. platensis* is a cosmopolitan species that can grow in temperate and tropical regions, so that it can be found in various lake ecosystems (Richmond, 2004; Soundarapandian and Vasanthi, 2008). Thengodkar and Sivakami (2010) reported that *A. platensis* has high tolerance to pesticides and this organisms can biodegrade these compounds. For this reason, it is an important organism for determining the pesticides concentrations that living things can survive. Also, it can also be easily produced under laboratory conditions (Vonshak and Tomaselli, 2000).

It is known that pesticides produce oxidative stress and the organisms react with antioxidant defense systems. The changes in the activity of the antioxidant defense system in the cyanobacteria under oxidative stress reflect the degree of tolerance and sensitivity to the pesticide which is exposed. However, the information on interactions between cyanobacterial antioxidant defense system and changing environmental conditions is limited (Mallick and Mohn, 2000). For this purpose, we focus on three key enzymes that are sensitive to pesticide stress on *A. platensis*.

Superoxide dismutase (SOD: EC 1.15.1.1) is a metalloenzyme that catalyzes superoxide to molecular oxygen and H_2O_2 (Valentine *et al.*, 1998). Ascorbate peroxidase catalyzes the reduction of hydrogen peroxide to water by using ascorbate as an electron donor. In parallel with APX activity, monodehydroascorbate (MDHA) and monovalent oxidant ascorbate are produced. MDHA spontaneously converts into ascorbic acid (AsA) and dehydroascorbate (DHA). DHA reductase produces ASA as oxidized dehydroascorbic acid (DAsA) using glutathione as an electron donor. Thus, the accumulation of toxic levels of H_2O_2 is prevented in photosynthetic organisms (Noctor and Foyer, 1998; Chew *et al.*, 2003). Glutathione reductase is an enzyme from the NADPH-dependent oxidoreductase family found in both prokaryotic and eukaryotic cells. GR catalyzes the reaction that allows conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) in conjunction with NADPH oxidation.

This study hypothesizes that Lambda cyhalothrin pesticide develops pesticide stress on aquatic organisms, inhibits growth and development, and develops the antioxidant defense mechanism in response to Lambda cyhalothrin pesticide. Also, the study is aimed to comprehend pesticide damages in the aquatic ecosystem via measuring the effects of Lambda cyhalothrin on phototrophic prokaryotes and to determine the oxidative stress responses and tolerance of cyanobacteria. Determination of oxidative stress will allow us to understand the effect of pesticides on *A. platensis* at the cellular level.

2 Materials and methods

2.1 Cyanobacteria culture and treatment

Arthrospira platensis M2 (SLSP01) strain obtained from Soley Microalgae Institute (California, USA) was cultured under axenic conditions in Spirulina Medium (18 g Na_2CO_3 , 1 g NaCO_3 , 1 g K_2HPO_4 , 2 g NaNO_3 , 1 g KSO_4 , 1 g NaCl , 0.4 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) (Aiba and Ogawa, 1977). Firstly cultures activated in the growth chamber (Biobase BJPX-A25011) and *A. platensis* cultures inoculated with different pesticide concentrations (0, 6.25, 12.5, 25, 37.5, 50, and 100 $\mu\text{g ml}^{-1}$) after the ten days. The culture conditions were 93 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ photosynthetically available radiation in 12:12 h light/dark cycle at 30 ± 1 °C for 7 days. The commercial formulation of Lambda Cyhalothrin (50 g L^{-1} , EC, Sakarya, Turkey) was used in all bioassay and prepared in distilled water. The range of concentrations was determined with preliminary range-finding bioassays according to EC50 value for growth parameters. Biological samplings were done with three replicate (Önem *et al.*, 2018).

2.2 Cell growth and chlorophyll-a assay

The optical density of cyanobacteria was measured in the spectrophotometer at 560 nm absorbance by dilution with 1/10 ratio. The change in OD was observed for 7 days, with each measurement being every 24 h. Thus, the growth rate was calculated. The diluted samples with methanol extracted for chlorophyll-a content and were measured spectrophotometrically (Shimadzu UVmini 1240 spectrophotometer) for 7 days (MacKinney, 1941). Because these methods are reliable and effective.

2.3 Antioxidant enzyme activities

At the end of the 7th day of study, 2 ml culture solutions from Lambda-cyhalothrin exposed cyanobacteria medium were centrifuged at 14.000 rpm for 20 min at 4 °C and pellets were stored at -20 °C until enzyme activity measurements. The grounded pellets with liquid nitrogen treated with in different buffers for each enzyme analysis. Bradford (1976) method was used for the total protein concentrations of cyanobacterial cell extracts and bovine serum albumin (BSA) was used as a standard.

Beyer and Fridovich (1987) method was used for total Superoxide dismutase (SOD; EC 1. 15. 1. 1) activity. Pellets were obtained from 2 ml samples after the centrifugation and then extracted with 1.5 ml homogenization buffer containing 1 mM Na_2EDTA , 100 mM K_2HPO_4 buffer (pH 7.0), and 2% PVP. Homogenates were centrifuged with 14.000 rpm for 20 min at 4 °C and supernatants were used for the assay. The reaction mixture prepared with 100 mM K_2HPO_4 buffer (pH 7.8) containing 5.7×10^{-5} M NBT, 9.9×10^{-3} M methionine, %1 tritonX-100, and enzyme extract. After 0.9 μM riboflavin was added and the mixture was exposed to light with an intensity of 375 $\mu\text{mole m}^{-2} \text{s}^{-1}$. At the end of this period, the absorbance values of 560 nm were read in spectrophotometer. SOD activity was calculated by a standard graphic and expressed as unit mg^{-1} protein.

Wang *et al.* (1991) method was used for Ascorbate peroxidase (APX; EC 1.11.1.11) activity and estimated the decreasing rate of ascorbate oxidation at 290 nm. Pellets were obtained from 2 ml samples after the centrifugation and then extracted with 1.5 ml homogenization buffer containing 2% PVP, 50 mM Tris-HCl (pH 7.2), 2 mM ascorbate and 1 mM Na₂EDTA, Homogenates were centrifuged with 14,000 rpm for 20 min at 4 °C and supernatants were used for the assay. 1 ml reaction solution prepared with 50 mM KH₂PO₄ buffer (pH 6.6), 2.5 mM ascorbate, 10 mM H₂O₂, and enzyme-containing 100 µg protein. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of ascorbate ($E = 2.8 \text{ mM cm}^{-1}$ at 290 nm).

Sgherri *et al.* (1994) method was used for Glutathione reductase (GR; EC 1.6.4.2) activity. Pellets were obtained from 2 ml samples after the centrifugation and then extracted with 1.5 ml of suspension solution containing, 1 mM Na₂EDTA, 2% PVP, and 100 mM KH₂PO₄ buffer (pH 7.0). 1 ml reaction solution prepared with 2 mM Na₂EDTA, 100 mM KH₂PO₄ buffer (pH 7.8), 0.2 mM NADPH, 0.5 mM oxidised glutathione (GSSG), and enzyme extract containing 100 µg protein. The decrease in absorbance at 340 nm was recorded. The correction was made for the non-enzymatic oxidation of NADPH by recording the decrease at 340 nm without adding GSSG to assay mixture. The enzyme activity was calculated from the initial rate of the reaction after subtracting the non-enzymatic oxidation using the extinction coefficient of NADPH ($E = 6.2 \text{ mM cm}^{-1}$ at 340 nm).

2.4 Statistical analysis

The differences between the control and treated samples were analyzed by one-way ANOVA, taking $p < 0.05$ as significant according to Tukey test with using SPSS 20.0 programme. EC50 value was determined with using Origin Pro 8.5 programme. Three replicate cultures were used for each treatment. The mean values \pm SE were given in Figures.

3 Results

When the effects of Lambda cyhalothrin from the first day to the seventh day were compared, there was a significant decrease in the amount of biomass and chlorophyll-*a* depending on the pesticide concentration ($p < 0.05$). The highest decrease in biomass and chlorophyll-*a* values between concentrations was found in 100 µg ml⁻¹ Lambda cyhalothrin application. (Fig. 1a, b).

The effect of Lambda cyhalothrin applications on SOD activity in *A. platensis*-M2 cells was presented in Figure 2a. According to our results, SOD activity increased at the most concentration by about 117% when compared to control ($p < 0.05$) on the other hand the changes of other concentrations are not significant ($p > 0.05$). APX activity was statistically different from the control at 100 µg ml⁻¹ Lambda cyhalothrin application (178% of control) in *A. platensis*-M2 cells ($p < 0.05$) (Fig. 2b). On the other hand, GR activity did not effect significantly ($p > 0.05$) (Fig. 2c).

4 Discussion

Lambda cyhalothrin which is a synthetic pyrethroid known to be dispersible in water have been various toxic effects in every step of the aquatic food chain. Zhang *et al.* (2011) reported that Lambda cyhalothrin pesticide had growth-limiting effects on *Chlorella vulgaris* and the LC50 value was found as 105.71 mg L⁻¹. Gupta and Baruah (2015) also observed that biomass and chlorophyll-*a* content of *Calothrix* sp. significantly decreased at 160 ppm Lambda cyhalothrin concentration (respectively %54.5 and %68). Mohapatra *et al.* (2003) showed that cypermethrin degraded the chlorophyll-*a* content of *Anabaena* sp. at 20 and 50 mM concentrations. Mohapatra *et al.* (2003) explained this situation based on Calow and Sibly's (1989) metabolic cost hypothesis. Accordingly, toxic substances trigger stress on the metabolic activities by creating stress in the organism and it causes a negative effect on the biochemical composition and leads to depletion of energy reserves. Reduced cyanobacterial growth with increasing pesticide concentrations may occur by the loss of carbon-assimilating photosynthetic pigments and damage to protein synthesis. In other words, the degradation of pigments, the main source of the production of the carbon skeleton, also prevents growth. The inhibitor effects of Lambda cyhalothrin on *Arthrospira platensis* were determined to be dose-dependent and it is explained with damage on chlorophyll-*a* content. These results are consistent with the harmful effects of other insecticides on chlorophyll-*a* and growth (Prasad *et al.*, 2005). The phytoplankton community exhibited high variability in their susceptibility to different pesticide concentrations and it is necessary for restructuring community models (Blanck, 2002).

Organic chemicals can be taken to their inner part by microorganisms and produce ROS such as superoxide radicals and hydrogen peroxide via taking part in biotransformation mechanisms (Vandana *et al.*, 2001). By the induction of SOD which is the first line of defense against the formation of toxic oxygen species, the cell system prevents damage caused by the superoxide radical therefore the activity of the enzyme increases (Lenártová *et al.*, 1998; Okamoto *et al.*, 2001). This increase depends on the duration and severity of stress (Okamoto *et al.*, 2001). Lin *et al.* (2009) have found that bensulfuron-methyl application stimulated to SOD activity of *Bacillus subtilis* B19, *B. megaterium* L1, and *Escherichia coli* K12. Kumar *et al.* (2014) emphasized SOD activity is also increased with the treatment of some herbicides in some bacterial cells. Kumar *et al.* (2014) observed SOD activity induced with chlorpyrifos application in *Chroococcus turgidus* NTMS12 cells. Li *et al.* (2005) reported that the alteration of SOD activity was susceptible to the surrounding area and it is affected by cypermethrin treatment. The other studies accentuated that the stimulation of SOD activity before growth and reproduction were arose from being at molecular levels in the cell (Rabinowich and Fridovich, 1985; Li *et al.*, 2005). In our study SOD activity increased accordingly control at the highest concentration (100 µg ml⁻¹). The studies have shown similar results to our results and suggest that pesticides have stimulatory effects on SOD enzyme activity.

APX uses the power of reducing ascorbic acid to remove potentially harmful H₂O₂ (Kumar *et al.*, 2008). Prasad *et al.* (2005)

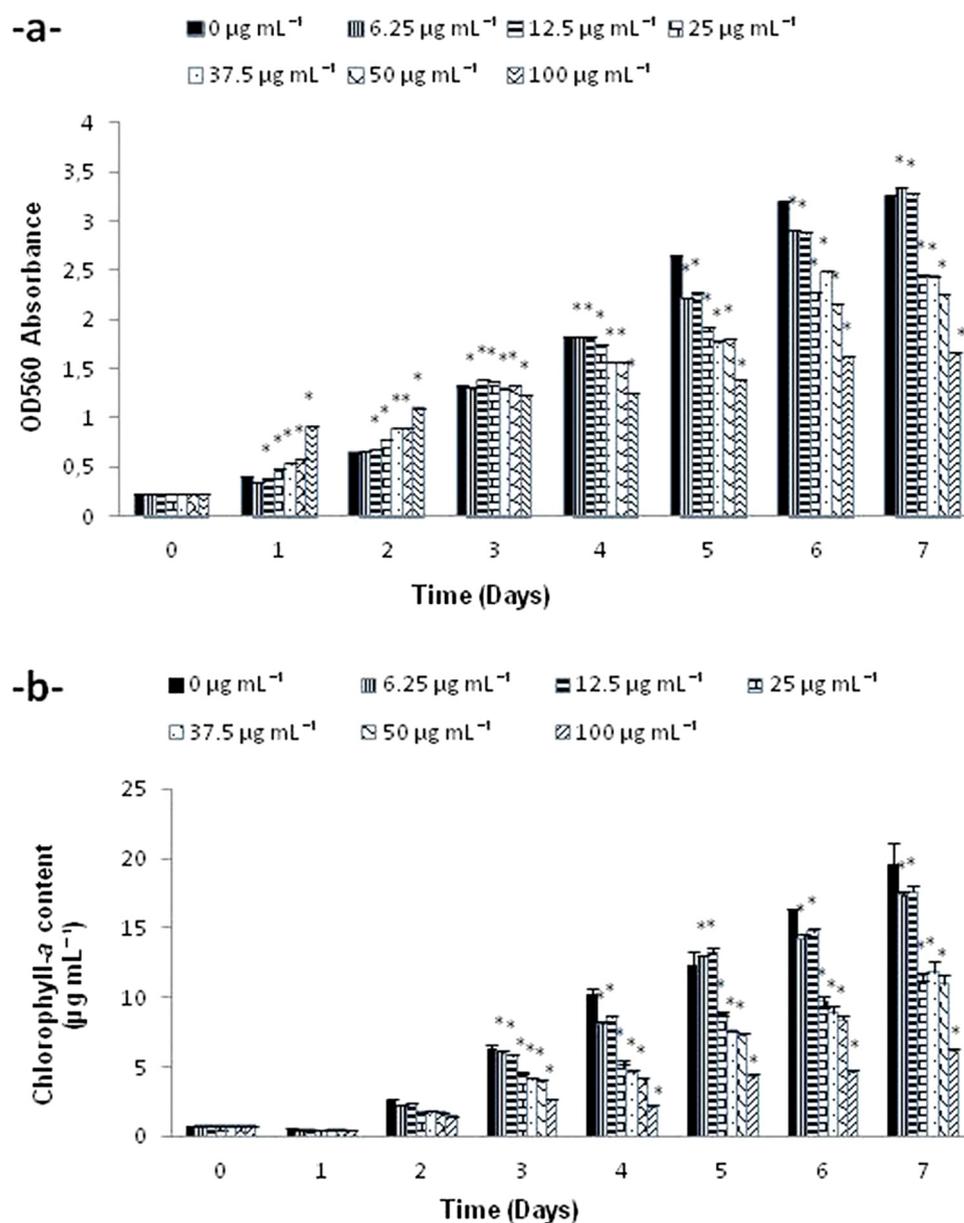


Fig. 1. Biomass values (a) and chlorophyll-*a* contents (b) of *A. platensis* supplemented with 0–100 µg mL⁻¹ Lambda cyhalothrin during 7 days. Data are the means ± SE of three replicates.

found that endosulfan increased the APX activity (50%) of *Plectonema boryanum* at 20 µg mL⁻¹ concentration. Bi *et al.* (2012) observed that SOD and APX activity of *Chlamydomonas reinhardtii* were increased together with isoprotruron application. Piotrowska-Niczyporuk and Bajguz (2014) studied on effects of auxins to *Chlorella vulgaris* and they found that these chemicals enhanced the activity of SOD and APX. In our results similar to SOD activity, 100 µg mL⁻¹ Lambda cyhalothrin application resulted in significantly higher APX activity (178% of control). The increase in APX activity correlates with the increase in SOD activity because SOD constitutes H₂O₂ as substrate of APX.

Dewez *et al.* (2005) found that fludioxonil enhanced the antioxidant activity but GR activity did not affect from this application on *Scenedesmus obliquus* cells and the similarly the application of Lambda cyhalothrin did not change the activity of GR in our results. Dewez *et al.* (2005) explained that APX activity used mostly oxidized glutathione pool.

In conclusion, our study results displayed that Lambda cyhalothrin concentrations in the culture medium affected the growth rate and chlorophyll-*a* content in *A. platensis*-M2 cells and these alterations of the growth rate and chlorophyll-*a* content were dose and time-dependent manner. Also, Lambda cyhalothrin caused oxidative stress in *A. platensis* cells.

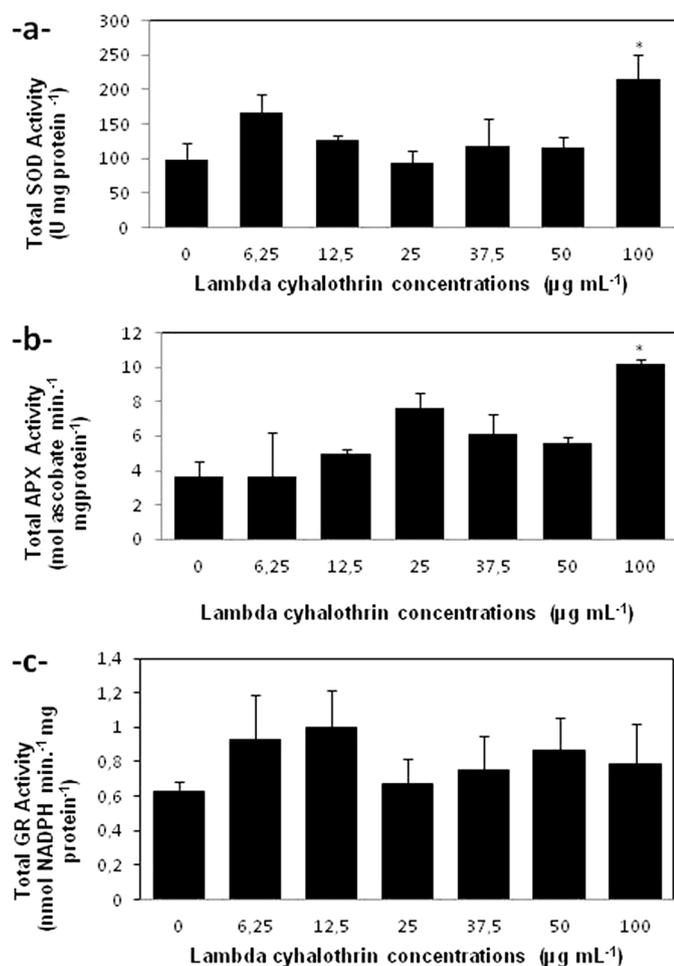


Fig. 2. Total SOD (a), APX (b) and GR (c) activities of *A. platensis* supplemented with 0–100 $\mu\text{g mL}^{-1}$ Lambda cyhalothrin. Data are the means \pm SE of three replicates.

As a result, Lambda cyhalothrin concentrations used in this study may be a step to prevent pesticide pollution in the environment.

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