ACTA BOTANICA CROATICA
CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

ACCEPTED, AHEAD OF PRINT VERSION OF THE MANUSCRIPT
80(2), 1st October 2021

Effects of two synthetic pyrethroids on Arthrospira platensis Gomont growth and antioxidant parameters

Hatice Tunca, Kübra Hödük, Feray Köçkar, Ali Doğru, Tuğba Ongun Sevindik

Please cite this article as: Tunca H, Hödük K, Köçkar F, Doğru A, Sevindik TO: Effects of two synthetic pyrethroids on Arthrospira platensis Gomont growth and antioxidant parameters. Acta Botanica Croatica, 10.37427/botcro-2021-011

Effects of two synthetic pyrethroids on *Arthrospira platensis* Gomont growth and antioxidant parameters

Hatice Tunca[1*], Kübra Hödük[1], Feray Köçkar[2], Ali Doğru[1], Tuğba Ongun Sevindik[1]

[1] University of Sakarya, Faculty of Arts and Science, Department of Biology, Sakarya, Turkey
[2] University of Balıkesir, Faculty of Arts and Science, Department of Molecular Biology and Genetics, Balıkesir, Turkey

*Corresponding author e-mail: htunca@sakarya.edu.tr

Running title: Oxidative damage from pesticide to *Arthrospira platensis*

Abstract – The transport of pesticides from application areas to other areas results in pesticide contamination and this sort of contamination has led to unexpected environmental problems worldwide. It is important to determine the responses of phytoplanktonic organisms to these chemicals for an understanding of the effects of pesticides on aquatic ecosystems. In this study, *Arthrospira platensis* Gomont cyanobacteria were exposed to different concentrations of the pesticides cypermethrin (0-50 µg mL^{-1}) and deltamethrin (0-2 µg mL^{-1}). Changes in chlorophyll-*a* concentration, the absorbance of OD560, antioxidant parameters (SOD, APX, GR, MDA, H_2O_2, and proline) were determined under the pesticide exposure. Our results showed that there is a decrease in OD560 absorbance and chlorophyll-*a* content proportionate to the increase of pesticide levels. Superoxide dismutase enzyme activity decreased with Cyp and Dlm application in *A. platensis* cultures. Glutathione reductase enzyme activity also decreased with Cyp applications but did not change with Dlm application. Ascorbate peroxidase enzyme activity increased with Cyp treatments but did not change with Dlm applications. Although malondialdehyde and hydrogen peroxide contents did not change with Cyp applications, they increased with Dlm applications. Proline contents increased with Cyp applications but decreased with Dlm applications. In conclusion, deltamethrin is more toxic than cypermethrin in the concentrations applied.

Keywords: antioxidant enzymes, *Arthrospira platensis*, cypermethrin, deltamethrin, growth

Introduction

Many chemicals, including pesticides, are released through modern industrial and agricultural activities and have reached a high level in the environment (Burkiewicz et al. 2005). Pesticides affect not only target organisms but also non-target organisms in the aquatic biota (Tremolada et al. 2004). These organisms play an important role in biological processes such as biogeochemical cycling, production, separation, and interaction with other organisms. Pesticides disturb the balance of water ecosystems with their direct action on plants and animals or with their bioaccumulation and transfer abilities in the food chain (Netrawali and Gandhi 1990, Burkiewicz et al. 2005). Planktonic algae display a fundamental role as primary producers (Burkiewicz et al. 2005) and a decrease in algal density and species composition affects the aquatic ecosystem directly by reducing biodiversity and primary production (Li et al. 2005). They are sensitive indicators that allow testing of the different effects of chemicals released into the water (Burkiewicz et al. 2005). For this reason, microalgae are frequently used in various

bioassays (Li et al. 2005). Agricultural chemicals inhibit the growth rate, biomass, and pigment content of freshwater algae by contaminating surface waters in agricultural areas (Netrawali and Gandhi 1990).

Pyrethroids are a class of synthetic insecticides designed and optimized based on pyrethrin structure (Elliott 1995). These pesticides are effective insecticides that are widely used to control agricultural and healthcare pests. After use, they are released into the environment and enter water resources (Mittal et al. 1994). Cypermethrin (Cyp) and deltamethrin (Dlm) have increased toxicity, especially to aquatic organisms with increasing life expectancy (Johri et al. 1997). Megharaj et al. (1987) observed that Cyp has inhibitory effects on *Scenedesmus bijugatus* Kützing. Xiong et al. (2002) and Li et al. (2005) found similar results for *Scenedesmus obliquus* Kützing. Wang et. al. (2011) carried out a growth inhibition test on *Skeletonema costatum* Cleve, *Scrippsiella trochoidea* (F.Stein) A.R.Loeblich III and *Chattonella marina* (Subrahmanyan) Y.Hara et M.Chihara during 96 h. Saenz et al. (2012) studied Cyp toxicity to *Scenedesmus quadricauda* Chodat, *Scenedesmus acutus* Meyen, *Chlorella vulgaris* Beyerinck (Beijerinck) and *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák. Wang et al. (2012) tested Cyp effetcs on *Scenedesmus obliquus*. These references show that Cyp has toxic effects on various groups of microalgae. There are also some studies on Dlm toxicity to aquatic microorganisms. Baeza-Squiban et al. (1987) have shown that the growth of *Dunaliella* sp. Teodoresco and *Chlamydomonas* sp. Ehrenberg was inhibited by Dlm application. Caquet et al. (1992) observed Dlm effects on phytoplankton communities in freshwater mesocosms and found Dlm to disappear rapidly from the aquatic ecosystem.

Antioxidants are compounds that reduce the harmful effects of oxidation via inhibiting free oxygen formation or eliminating formed free radicals (Baublis et al. 2000). Superoxide dismutase (SOD: EC 1.15.1.1) is a class of metalloprotein that catalyses superoxide to oxygen and hydrogen peroxide (H_2O_2) (Valentine et al. 1998). Ascorbate peroxidase (APX: EC 1.11.1.11) converts hydrogen peroxide to water by using ascorbate as an electron donor and thereby accumulations of H_2O_2 toxic levels are prevented in photosynthetic organisms (Chew et al. 2003). Glutathione reductase (GR: EC 1.6.4.2), is a member of NADPH-dependent oxidoreductases, found in both prokaryotic and eukaryotic cells. GR catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) together with the oxidation of NADPH and plays an important role in the cellular defence systems against reactive oxygen metabolites by creating a reduced GSH pool (Anjum 2010). Malondialdehyde is a metabolite that occurs with peroxidation of lipids including three or more double bonds. Lipid peroxidation causes malondialdehyde formation (Goel and Sheoran 2003). It is known that proline content increases under various stress factors and proline accumulation protects cell parts and cell contents such as enzymes, membranes and polyribosomes (Kishor et al. 2005).

The enzymatic and non-enzymatic antioxidant defence systems create the response to oxidative stress and reflect the tolerance and susceptibility of algae to pesticide exposure. However, although there is some information about algal antioxidant systems, which are either a response to the environmental conditions or a defensive system (Mallick and Mohn 2000) studies about the harmful or activator effects of pyrethroids on algal antioxidant systems are limited.

The aims of our study are: (i) to determine metabolic damage due to cypermethrin and deltamethrin toxicity and (ii) to measure the intracellular level of antioxidant responses for pesticide detoxification in *Arthrospira platensis*-M2 strain**.** For this purpose, the alterations of some parameters such as growth rate (OD560), chlorophyll-*a* content, superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline have been investigated during pesticide application. Responses given by phototrophic microorganisms to pesticides will help us to have an idea of how the communities are affected by the pesticides in the water ecosystems.

Material and methods

Algae culture and treatment

Arthrospira platensis, strain M2, was obtained from the Soley Microalgae Institute (California, USA) (Culture collection No: SLSP01). Algae were grown in Spirulina Medium (Aiba and Ogawa 1977) under axenic conditions. 20 mL algal cultures were inoculated onto 180 mL culture medium in an Erlenmeyer flask and were allowed to grow under full-spectrum lamps providing 93 µmol photons m^{-2} s^{-1} photosynthetically available radiation in a 12:12 h light/dark cycle at 30 ± 1 ºC during 10 days. At the end of 10 days, cultures were renewed and, all the flasks contained 50 mL algal culture. Stock solutions of the commercial formulation of Cyp and Dlm (200 g L^{-1} and 25 g L^{-1}, respectively; EC, Sakarya, Turkey) were prepared with distilled water and were then diluted for all bioassays. Various concentrations of cypermethrin (10, 20, 30, 40, 50 µg mL^{-1}) and deltamethrin (0.125, 0.25, 05, 1, 1.5, 2 µg mL^{-1}) were added to the culture medium. The range of concentrations was determined with preliminary range-finding bioassays according to EC50 value for growth parameters and IC50 value for enzyme activity assays and at least five concentrations were selected under these values.

Cell growth and chlorophyll-*a* assay

Optical densities (ODs) of microalgae were measured spectrophotometrically over 7 days under control and stressed conditions taking absorbance at 560. Chlorophyll-*a* content was estimated by methanol extraction and measured spectrophotometrically over 7 days (MacKinney 1941).

Antioxidant enzyme activities

On the 7^{th} day of the study, 2 mL culture solutions from the control and treated samples were centrifuged at 14.000 rpm for 20 min at 4 °C and the resulting pellets were kept at -20 °C until enzyme activity was measured. Pellets were ground with liquid nitrogen and suspended in specific buffers with proper pH values for each enzyme. The protein concentrations of algal cell extracts were determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard.

The superoxide dismutase activity was determined by the method of Beyer and Fridovich (1987), based on the photoreduction of NBT (nitroblue tetrazolium). Extraction of pellets (0.2 g) was performed in 1.5 mL homogenization buffer containing 100 mM K_2HPO_4 buffer (pH 7.0), 2% PVP, and 1 mM Na_2EDTA. After centrifugation at 14,000 rpm for 20 min at 4 °C, the resulting supernatants were used to measure SOD activity. The reaction mixture consisted of 100 mM K_2HPO_4 buffer (pH 7.8) containing 9.9×10^{-3} M methionine, 5.7×10^{-5} M NBT, %1 triton X-100, and enzyme extract. The reaction was started by the addition of 0.9 µM riboflavin and the mixture was exposed to light with an intensity of 375 µmole m^{-2} s^{-1}. After 15 min, the reaction was stopped by switching off the light, and absorbance was read at 560 nm. The SOD activity was calculated by a standard graphic and expressed as U mg^{-1} protein.

The ascorbate peroxidase activity was determined according to Wang et al. (1991) by estimating the decreasing rate of ascorbate oxidation at 290 nm. APX extraction was performed in 50 mM Tris–HCl (pH 7.2), 2% PVP, 1 mM Na_2EDTA, and 2 mM ascorbate. The reaction mixture consisted of 50 mM K_2HPO_4 buffer (pH 6.6), 2.5 mM ascorbate, 10 mM H_2O_2, and enzyme-containing 100 µg protein in a final volume of 1 mL. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of ascorbate (E = 2.8 mM cm^{-1} at 290 nm).

The glutathione reductase activity was measured with the method of Sgherri et al. (1994). Extraction was performed in 1.5 mL of suspension solution containing 100 mM K_2HPO_4 buffer

(pH 7.0), 1 mM Na_2EDTA, and 2% PVP. The reaction mixture (total volume of 1 mL) contained 100 mM K_2HPO_4 buffer (pH 7.8), 2 mM Na_2EDTA, 0.5 mM oxidised glutathione (GSSG), 0.2 mM NADPH and enzyme extract containing 100 µg protein. The decrease in absorbance at 340 nm was recorded. 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 mM cm^{-1} at 340 nm).

Determination of malondialdehyde and hydrogen peroxide

The malondialdehyde content was determined by the method of Heath and Packer (1968). 0.2 g of pellet was homogenized in 3 mL of 0.1% TCA (4 °C) and centrifuged at 4100 rpm for 15 min and the supernatant was used in the subsequent determination. 0.5 mL of 0.1 M Tris–HCl pH 7.6 and 1 mL of TCA–TBA–HCl reagent (15% w/v) (trichloroacetic acid–0.375% w/v thiobarbituric acid–0.25 N hydrochloric acid) were added to the 0.5 mL of the supernatant. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. To remove suspended turbidity, the mixture was centrifuged at 4100 rpm for 15 min, then the absorbance of the supernatant at 532 nm was recorded. Non-specific absorbance at 600 nm was measured and subtracted from the readings recorded at 532 nm. The MDA content was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}. For determination of the hydrogen peroxide content, 0.5 mL of 0.1 M Tris–HCl (pH 7.6) and 1 mL of 1 M KI were added to 0.5 mL of supernatant. After 90 min, the absorbance was recorded at 390 nm.

The proline content determination

The proline content was determined by the method of Weimberg et al. (1982). 0.1 g of pellet was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and the homogenates were incubated in a hot water bath at 95 ^oC for 30 minutes. The samples were cooled and centrifuged at 4100 rpm for 10 min. Two mL of the extract reacted with 2 mL of acid–ninhydrine and 2 mL of glacial acetic acid for 1 h at 100°C. The reaction mixture was extracted with 4 mL toluene. The chromophore containing toluene was separated and the absorbance was recorded at 520 nm.

Statistical analysis

The differences between the means of control and treated samples were analysed by one-way analysis of variance (ANOVA), taking $P < 0.05$ as significant according to LSD with two degrees of freedom. Three replicate cultures were used for each treatment. The mean values ± SE are shown in Figures.

Results

Biomass and chlorophyll-*a* content

Biomass production and chlorophyll-*a* content of *Arthrospira platensis*-M2 cells were significantly decreased by Cyp and Dlm application to the culture medium for 7 days ($P < 0.05$, Figs. 1, 2). The most significant reductions were observed at the highest pesticide treatments, (50 µg mL^{-1} for Cyp and 2 µg mL^{-1} for Dlm) in biomass production and chlorophyll-*a* content. Cyp and Dlm toxicity affected the biomass production and chlorophyll-*a* content of *A. platensis*-M2 cells, which increased in a time-dependent manner during the 7 days (Figs. 1a,b, 2a,b). The EC 50 values of Cyp and Dlm applications are 53.12 ± 1.16 µg mL^{-1} and 1.76 ± 0.07 µg mL^{-1} respectively for 7^{th} day, based on biomass production.

The antioxidant parameters

The effect of Cyp applications on SOD activity in *A. platensis*-M2 cells is presented in Fig. 3a. All Cyp concentrations (5, 10, 20, 30, 40, 50 µg mL^{-1}) caused an increase in SOD activity on *A. platensis*-M2 cells as compared to that of control. The maximum inhibition in SOD activity (96%) occurred at 40 µg mL^{-1} Cyp concentration. APX activities increased at the same concentrations ($P < 0.05$, Fig. 3b). On the other hand GR activities also decreased at all concentrations except 5 µg mL^{-1} Cyp application ($P < 0.05$, Fig. 3c). Ten, 20, 30, 40, 50 µg mL^{-1} Cyp concentrations caused to decrease by 17, 66, 47, 70, 85% in GR activity, respectively. MDA and H_2O_2 contents (Fig. 4a,b) did not change statistically but proline contents (Fig. 4c) increased at all Cyp concentrations ($P > 0.05$).

The effect of Dlm applications on SOD activity in *A. platensis*-M2 cells is presented in Fig. 5a. SOD activities decreased by 37, 47, 21, 39, 36, 52% ($P < 0.05$) at all the Dlm concentrations, respectively; APX and GR activities did not display any alteration statistically ($P > 0.05$, Fig. 5b,c). MDA contents increased at 0.5, 1, 1.5 µg mL^{-1} Dlm applications (74, 85, 78%; respectively) ($P < 0.05$, Fig 6a); however H_2O_2 content increased with 1, 1.5 and 2 µg mL^{-1} Dlm applications ($P < 0.05$, Fig. 6b). Proline content decreased statistically at 2 µg mL^{-1} Dlm concentration ($P < 0.05$, Fig. 6c).

Discussion

In this study, we have found that exogenous addition of different concentrations of two synthetic pyrethroids (cypermethrin and deltamethrin) showed varying degrees of toxicity to the growth of Arthrospira platensis M2 strain. In a study of Scenedesmus bijugatus, Nostoc linckia Bornet ex Bornet et Flahault, Synechococcus elongatus (Nägeli) Nägeli and Phormidium tenue Gomont, Megharaj et al. (1987) found that cypermethrin inhibited the growth of Scenedesmus bijugatus at 10-50 µg mL−1 concentrations. Xiong et al. (2002) applied cypermethrin to Scenedesmus obliquus during a 96 h period and found that the EC50 was 112 mg L-1. Li et al. (2005) reported that cypermethrin inhibited the growth of Scenedesmus obliquus at 50-250 mg L-1 concentrations during 96 h, and the chlorophyll-a and carotenoid content of Scenedesmus obliquus decreased with the application. Li et al. found the EC50 value of cypermethrin as 112 ± 9 mg L-1. Wang et. al. (2011) carried out a growth inhibition test about cypermethrin toxicity on Skeletonema costatum, Scrippsiella trochoidea, and Chattonella marina during 96 h and they determined EC50 values as 71.4 µg L−1, 205 µg L−1 and 191 µg L−1, respectively. Saenz et al. (2012) study cypermethrin toxicity to Scenedesmus quadricauda, Scenedesmus acutus, Chlorella vulgaris and Pseudokirchneriella subcapitat and the inhibited growth concentrations are 0.3-5 mg L-1, 0.6 mg L-1, 0.15 - 2.5 mg L-1 and 0.075 mg L-1, respectively. In another study, Wang et al. (2012) determine the toxic effects of commercial cypermethrin on S. obliquus and measure EC50 value of 2.37 mg L-1. The data and the concentrations used (10-50 µg mL-1) of Megharaj et al. (1987) are the closest to the results of our study. As was shown in the literature, Cyp has an inhibitory effect on algal growth and chlorophyll-a content.

As with the toxicity of Cyp, there are some studies about the effect of Dlm on algae. In the study of Burkiewicz et al. (2005), the cell density of Scenedesmus subspicatus Chodat decreased with 2.5, 5 and 10 mg L-1 Dlm applications during 24 h and 1.25 mg L-1 Dlm application during 72 h. Lutnicka et al. (2014) observed that Dlm has inhibited the growth of Chlorella vulgaris by 13% at the end of 14 days with a 0.02 µg L-1 Dlm application.

Although the Dlm concentrations in the study of Lutnicka et al. (2014) were lower than in our applications, the concentrations of Burkiewicz et al. (2005) were similar to those in our studies'.

In our study, SOD activities of A. platensis decreased at all Cyp applications except 5 µg mL-1 and at all Dlm applications according to control. In our study, Cyp and Dlm pesticides

also caused significant reductions in chlorophyll-a, and thus loss of photosynthetic metabolism may have caused significant reductions in SOD activity. Wang et al. (2011) also reported that Cyp pesticide in high concentrations (> 50 µg L-1) inhibited SOD activity in Skeletonema costatum, Scrippsiella trochoidea and Chattonella marina, and they pointed out the inactivation of SOD activity may have been due to growth being inhibited by cypermethrin. One or more of the suggested reasons in these articles may be the main mechanism of Cyp and Dlm toxicity on the SOD enzyme activity in our study.

GR and glutathione are effective on the Halliwell / Asada pathway in the inactivation of H2O2 in plant cells (Bray et al. 2000). GR catalyzes the last step of the ascorbate-glutathione pathway. GR activity significantly decreased at 10, 20, 30, 40, and 50 µg mL-1 Cyp applications. Saenz et al. (2012) found that Cyp concentrations causing algicidal effects, and thereby inhibitory effects of GR enzyme activity due to oxidative stress damage on P. subcapitata. The reductions in GR enzyme activities may have been related to loss of cell viability or deterioration of enzyme structure and enzyme reactions.

In contrast, GR activity did not change in Dlm application in our study. Dewez et al. (2005) treated a fungicide Fludioxonil to Scenedesmus obliquus and indicated that GR activity was not significantly affected and the oxidized glutathione pool may have also been used by other enzymes.

APX enzyme activity statistically increased at all concentrations in Cyp treatment. APX use ascorbic acid for the elimination of detrimental H2O2 (Verma and Dubey 2003). Studies on plants showed that APX activity increased in various stress conditions (Hideg and Vass 1996, Verma and Dubey 2003).

Teisseire and Vernet (2001) supported the conclusion that GR enzyme activity was related to the APX enzyme activity. In our study, it was concluded that the absence of alteration in GR activity at the application concentrations of Dlm promoted the absence of alteration in APX enzyme at similar concentrations because the ascorbate pool was counterbalanced by the GR enzyme.

In our study, MDA content in A. platensis cultures treated with Dlm significantly increased at 0.5 µg mL-1, 1 µg mL-1, and 1.5 µg mL-1 concentrations. The changes in MDA content were parallel with the changes of H2O2 content for Dlm pesticide. The increase of H2O2 content caused the formation of OH radicals by the Haber-Weis reaction and therefore lipid peroxidation was increased (Goel and Sheoran 2003). Moreover, the inhibition of SOD activity caused accumulation of O2- in the medium in deltamethrin application. It is known that lipid peroxidation is linked to the amount of O2- (Choudhary et al. 2007). Also, Baruah and Chaurasia (2020) reported that alpha-cypermethrin decreased the photosynthetic pigment content and it increased the MDA content of Chlorella sp. at 6-48 mg L-1 during 96 h bioassay. The reduction of photosynthetic pigments also increases lipid peroxidation (Chen et al. 2008). A significant reduction of chlorophyll-a content may take place with lipid peroxidation at Dlm application. It was reported that MDA content, an indicator of lipid peroxidation, was increased by the endosulfan application (Kumar et al. 2008). Wang et al. (2011) found that cypermethrin enhanced MDA content on Skeletonema costatum, Scripsiella trochoidea and Chatonella marina.

There was no significant change in the MDA content at the Cyp application and it was supported by the results of H2O2 content. Moreover, the increase in the proline content may have prevented the cell membrane damage and the amount of MDA. Siripornadulsil et al. (2002) reported that the cadmium application did not alter the amount of MDA in the proline overproducing transgenic Chlamydomonas reinhardtii P.A.Dangeard, and they suggested that proline may have inhibited free radical damage due to its antioxidant action.

The amount of H2O2 in A. platensis cultures exposed to the Dlm significantly increase with addition of 1 µg mL-1, 1.5 µg mL-1, and 2 µg mL-1 Dlm. It was expected that the

application of Dlm pesticide would result in a decrease in the H2O2 content with the increase of SOD enzyme activity, but the H2O2 content likely increased due to the increased activity of oxidases such as glycolate oxidase, glucose oxidase, amino acid oxidase and sulfite oxidase in plants (Asada 1999).

The amount of H2O2 in all concentrations of cultures of A. platensis exposed to Cyp (except 1 µg mL-1 and 5 µg mL-1) was not changed by increased APX enzyme activity. The decrease in the amount of H2O2 is likely due to the increase in enzymes consuming the H2O2 content, such as the APX enzyme (Mallick and Mohn 2000).

The free proline amount of A. platensis cultures exposed to Cyp was statistically significantly increased at all concentrations compared to the control. Proline accumulation has been reported in plants exposed to heavy metal stress (Saradhi and Saradhi 1991). Proline is an effective singlet oxygen scavenger and regulates the cell redox potential (Saradhi and Saradhi 1991, Alia et al. 2001). Proline acts as an osmotic regulator and scavenger of OH radical in cells and thus it interacts with cell macromolecules such as DNA, protein, and lipids and consequently stabilizes the structure and function of these molecules (Kavir et al. 2005). The increase of proline content may be an adaptive response under extreme stress conditions (Fatma et al. 2007, Kumar et al. 2014). Proline reduces free radical production under stress conditions (Alia Saradhi 1993). Fatma et al. (2007) studied the effects of environmental pollution by evaluating proline content on Westiellopsis prolifica Janet cyanobacterium and found that alphamethrin pesticide and heavy metals increased proline accumulation.

The free proline content of A. platensis cultures exposed to the Dlm effect was significantly reduced at 2 µg mL-1 concentration. Ewald and Schlee (1983) found that sulfide decreased the proline content by inhibiting proline synthesis. Likewise, Dlm may have inhibited the proline synthesis or may have disrupted proline configuration in our Dlm application.

In conclusion, in this study, the decrease in biomass and chlorophyll-a was related to the concentration of Cyp and Dlm. Antioxidant enzyme activities and parameters were affected by different degrees according to the particular pesticides and their concentrations. These differences arose from the ROS producing capacity of Cyp and Dlm. Also, deltamethrin is more toxic than cypermethrin according to concentrations. Since these pesticides have a toxic effect on aquatic organisms, care should be taken when they are used with all necessary precautions to prevent their release into the aquatic ecosystem.

Acknowledgement

This study was supported by Sakarya University Research Projects under Grant no. FBDTEZ 2014-50-02-014.

References

Aiba, S., Ogawa, T., 1977: Assessment of growth yield of a blue-green alga: *Spirulina platensis* in axenic and continuous culture. Journal of General Microbiology 102, 179–182.

Alia, S.P.P., 1993: Suppression in mitochondrial electron transport is the prime cause behind stress induced proline accumulation. Biochemical and Biophysical Research Communications 193, 54–58.

Alia, S.P.P., Mohanty, P., Matysik, J., 2001: Effect of proline on the production of singlet oxygen. Amino Acid 21, 195–200.

Anjum, N.A., Umar, S., Chan, M.T., 2010: Ascorbate-glutathione pathway and stress tolerance in plants. In: Pang, C.H., Wang, P.S. (eds.), Role of ascorbate peroxidase and glutathione reductase, 91–115. Springer Science, New York.

Asada, K., 1999: The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Physiology and Plant Molecular Biology 50, 601–639.

Baeza-Squiban, A., Marano, F., Ronot, X., Adolphe, M., Puiseux-Dao, S., 1987: Effects of deltamethrin and its commercial formulation DECIS on different cell types in vitro: Cytotoxicity, cellular binding, and intracellular localization. Pesticide Biochemistry and Physiology 28, 103–113.

Baruah, P., Chaurasia, N. 2020: Ecotoxicological effects of alpha-cypermethrin on freshwater alga *Chlorella* sp.: Growth inhibition and oxidative stress studies. Environmental Toxicology and Pharmacology 76, 103347.

Baublis, A.J., Clydesdale, F.M., Decker E.A., 2000: Antioxidants in wheat-based breakfast cereals. Cereals Foods World 45, 71–74.

Beyer, W.F., Fridovich, I., 1987: Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Analytical Biochemistry 161, 559–566.

Bradford, M.M.A., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.

Bray, E.A., Bailey-Serres, J., Weretilnyk, E., 2000: Biochemistry and Molecular Biology of Plants. In: Gruissem, W., Jones, R. (eds.), Responses to abiotic stress, 1158–1203. American Society of Plant Physiologists, Rockville.

Burkiewicz, K., Synak, R., Tukaj, Z., 2005: Toxicity of three insecticides in a standard algal growth inhibition test with *Scenedesmus subspicatus.* Bullettin of Environmental Contamination and Toxicolology 74, 1192–1198.

Caquet, T., Thybaud, E., Le Bras S., Jonot, O., Ramade F., 1992: Fate and biological effects of lindane and deltamethrin in freshwater mesocosms. Aquatic Toxicology 23, 261–277.

Chen, T.F., Zheng, W.J., Wong, Y.J. Yang, F., 2008: Selenium-induced changes in activities of antioxidant enzymes and content of photosynthetic pigments in *Spirulina platensis*. Journal of Integrative Plant Biology 50, 40–48.

Chew, O., Whelan, J., Millar, A.H., 2003: Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. The Journal of Biological Chemistry 278, 46869–46877.

Choudhary, M., Jetley, U. K., Khan, M. A., Zutshi, S., & Fatma, T. 2007: Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicology and environmental safety, 66(2), 204-209.

Dewez, D., Geoffroy, L., Vernet, G., Popovic, R., 2005: Determination of photosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga *Scenedesmus obliquus*. Aquatic Toxicology 74, 150–159.

Elliott, M., 1995: Chemicals in insect control. In: Casida, J.E., Quistad G.B. (eds.), Pyrethrum flowers: production, chemistry, toxicology, and uses, 3–31. Oxford University Press, New York.

Ewald, D., Schlee, D., 1983: Biochemical effects of sulphur dioxide on proline metabolism in the alga *Trebouxia* sp. New Phytology 94, 235–240.

Fatma, T., Khan, A., Choudhary, M., 2007: Impact of environmental pollution on cyanobacterial proline content. Journal of Applied Phycology 19, 625–629.

Goel, A., Sheoran, I.S., 2003: Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. Biologia Plantarum 46, 429–434.

Hideg, E., Vass, I., 1996: UV-B induced free radical production in plant leaves and isolated thylakoid membranes. Plant Science 115, 251–260.

Heath, R.L, Packer, L., 1968: Photoperoxidation in isolated chloroplasts. I. Stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125, 189–198.

Johri, A.K., Saxena, D.M., Lal, R., 1997: Interaction of synthetic pyrethroids with micro-organisms: a review. Microbios 89, 151–156.

Kavir, K.P.B., Sangam, S., Amrutha, R.N., Laxmi, P.S.N.K.R., 2005: Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science 88(3), 424-438.

Kishor, P. K., Sangam, S., Amrutha, R.N., Laxmi, P. S., Naidu, K.R., Rao, K.S., Sreenath Rao, Reddy, K.J., P. Theriappan P., Sreenivasulu, N., 2005: Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Current Science, 88, 424-438.

Kumar, S., Habib, K., Fatma, T. 2008: Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. Science of the Total Environment, 403(1-3), 130-138.

Kumar, S., Praveenkumar, R., Jeon, B.H., Thajuddin, N., 2014: Chlorpyrifos-induced changes in the antioxidants and fatty acid compositions of *Chroococcus turgidus* NTMS12. Letters of Applied Microbiology 59 (5), 535–541

Li, X., Ping, X., Xiumei, S., Zhenbin, W., Liqiang, X., 2005: Toxicity of cypermethrin on growth, pigments, and superoxide dismutase of *Scenedesmus obliquus*. Ecotoxicology and Environmental Safety 60, 188–192.

Lutnicka, H., Fochtman, P., Bojarski, B., Ludwikowska, A., Formicki, G., 2014: The influence of low concentration of cypermethrin and deltamethrin on phyto- and zooplankton of surface waters. Folia Biologica 62, 251–257.

MacKinney, G., 1941: Absorption of light by chlorophyll solution. The Journal of Biological Chemistry 140, 315–322.

Mallick, N., Mohn, F.H., 2000: Reactive oxygen species: response of algal cells. Journal of Plant Physiology 157, 183–193.

Megharaj, M., Venkateswarlu, K., Rao, A.S., 1987: Influence of cypermethrin and fenvalerate on a green alga and three cyanobacteria isolated from soil. Ecotoxicology and Environmental Safety 14, 142–146.

Mittal, P.K., Adak, T., Sharma, V.P., 1994: Comparative toxicity of certain mosquitocidal compounds to larvivorous fish, *Poecilia reticulata*. Indian Journal of Malariology 31, 43–47.

Netrawali, M.S., Gandhi, S.R., 1990: Mechanism of cell destructive action of organophosphorus insecticide phosalone in *Chlamydomonas reinhardtii* algal cells. Bulletin of Environmental Contamination and Toxicology 44, 819–825.

Sáenz, M.E., Marzio, W.D.D., Alberdi, J.L., 2012: Effects of a commercial formulation of cypermethrin used in biotech soybean crops on growth and antioxidant enzymes of freshwater algae. Journal of Environmental Protection 2, 15–22.

Saradhi, A., Saradhi, P.P., 1991: Proline accumulation under heavy metal stress. Journal of Plant Physiology 138, 554–558.

Sgherri, C.L.M, Loggini B., Puliga S., Navari-Izzo F., 1994: Antioxidant system in *Sporobolus stapfianus*: changes in response to desiccation and rehydration. Phytochemistry 35, 561–565.

Siripornadulsil, S., Traina, S., Verma, D.P.S., Sayre, R.T., 2002: Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. Plant Cell 14, 2837–2847.

Teisseire, H., Vernet, G., 2001: Effects of the fungicide folpet on the activities of antioxidative enzymes in duckweed (*Lemna minor*). Pesticide Biochemistry and Physiology 69, 112–117.

Tremolada, P., Finizio, A., Villa, S., Gaggi, C., Vighi, M., 2004: Quantitative inter-specific chemical activity relationships of pesticides in the aquatic environment. Aquatic Toxicology 67, 87–103.

Valentine, W.M., Amarnath, V., Amarnath, K., Erve, J.C.L., Graham, D.G., Morgan, D.L., Sills, R.C., 1998: Covalent modification of hemoglobin by carbon disulfide: a potential biomarker of effect. Neurotoxicology 19, 99–108.

Verma, S., Dubey, R.S., 2003: Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science 164, 645–650.

Wang, S.Y., Jiao, H., Faust, M., 1991: Changes in ascorbate, glutathione and related enzyme activity during thidiazuron-induced bud break of apple. Physiology Plantarum 82, 231–236.

Wang, Z., Xie, J., Jiang, S., Shi, J., Liu, Y., Gong, W., 2012: Effects of commercial cypermethrin on the growth of *Scenedesmus obliquus* and its physiochemical responses. China Environmental Science 32, 659–665.

Wang, Z.H., Nie, X.P., Yue, W.J., 2011: Toxicological effects of cypermethrin to marine phytoplankton in a co-culture system under laboratory conditions. Ecotoxicology 20, 1258–1267.

Weimberg, R., Lerner, H.R, Poljakoff-Mayber, A., 1982: A relationship between potassium and proline accumulation in salt-stressed *Sorghum bicolor*. Physiology Plantarum 55, 5–10.

Xiong, L., Wu, Z., Kuang, Q., Xia, Y., He, F., 2002: Studies on the toxicity of cypermethrin to *Scenedesmus obliquus*. Acta Hydrobiologica Sinica 26, 66–73.

Figure Legends

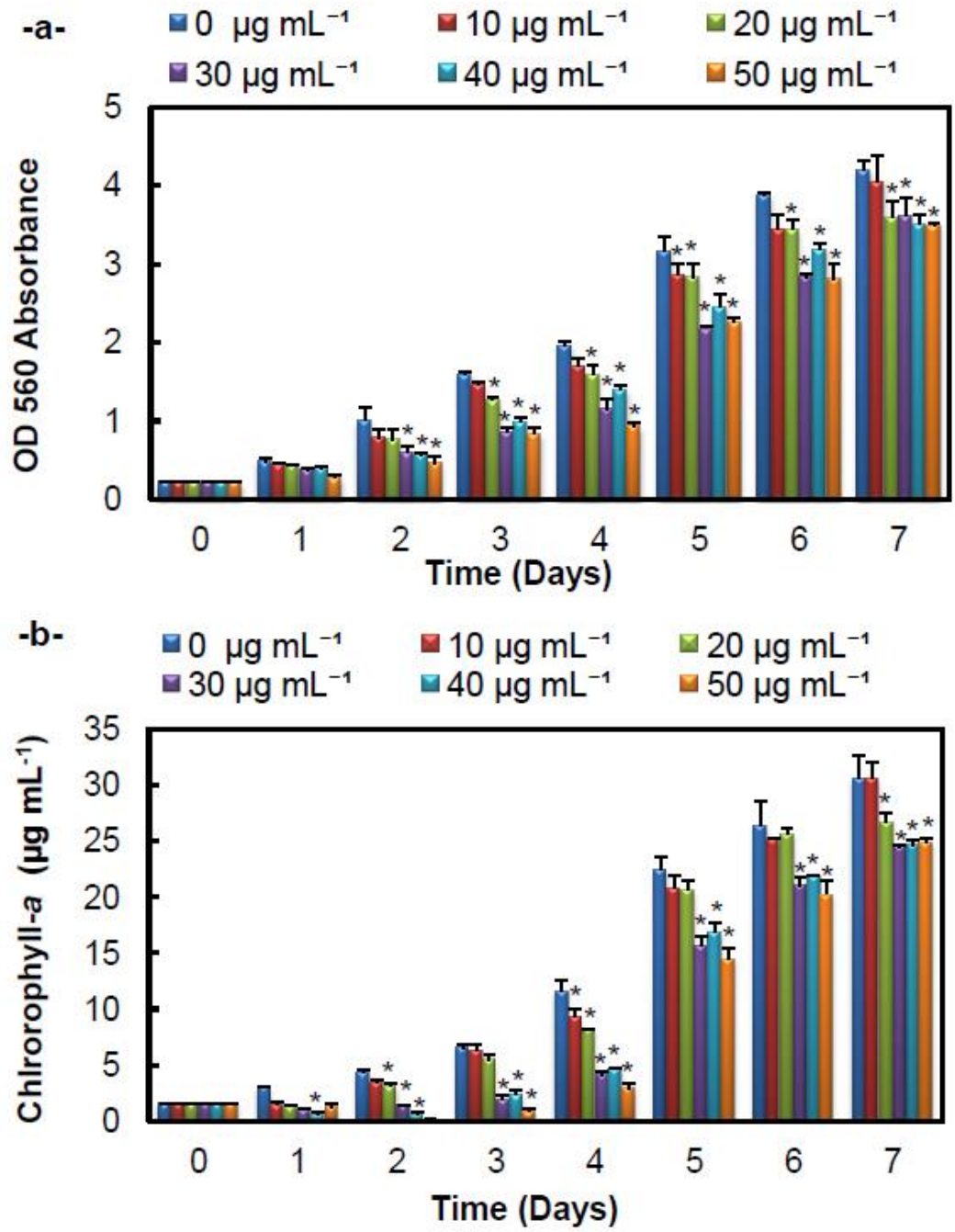

Fig. 1. Biomass values, as absorbance at 560 nm (a), and chlorophyll-*a* concentration (b) of *Arthrospira platensis* treated with 0-50 µg mL^{-1} cypermethrin during 7 days. Data are the means ± SE of three replicates. *Significantly different from control, $P < 0.05$ (LSD analysis).

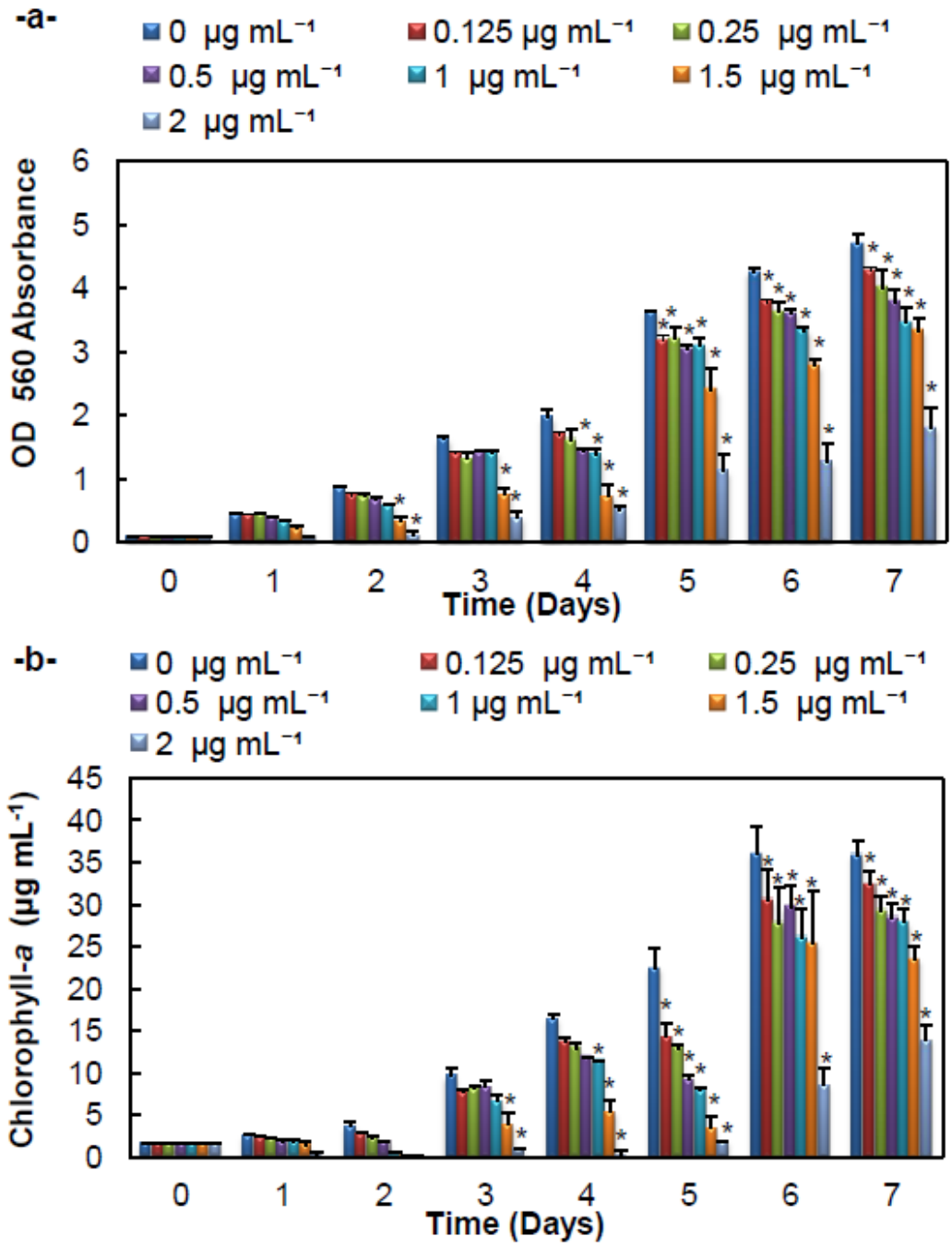

Fig. 2. Biomass values, as absorbance at 560 nm (a), and chlorophyll-*a* concentration (b) of *Arthrospira platensis* treated with 0-2 µg mL^{-1} deltamethrin during 7 days. Data are the means ± SE of three replicates. *Significantly different from control, $P < 0.05$ (LSD analysis).

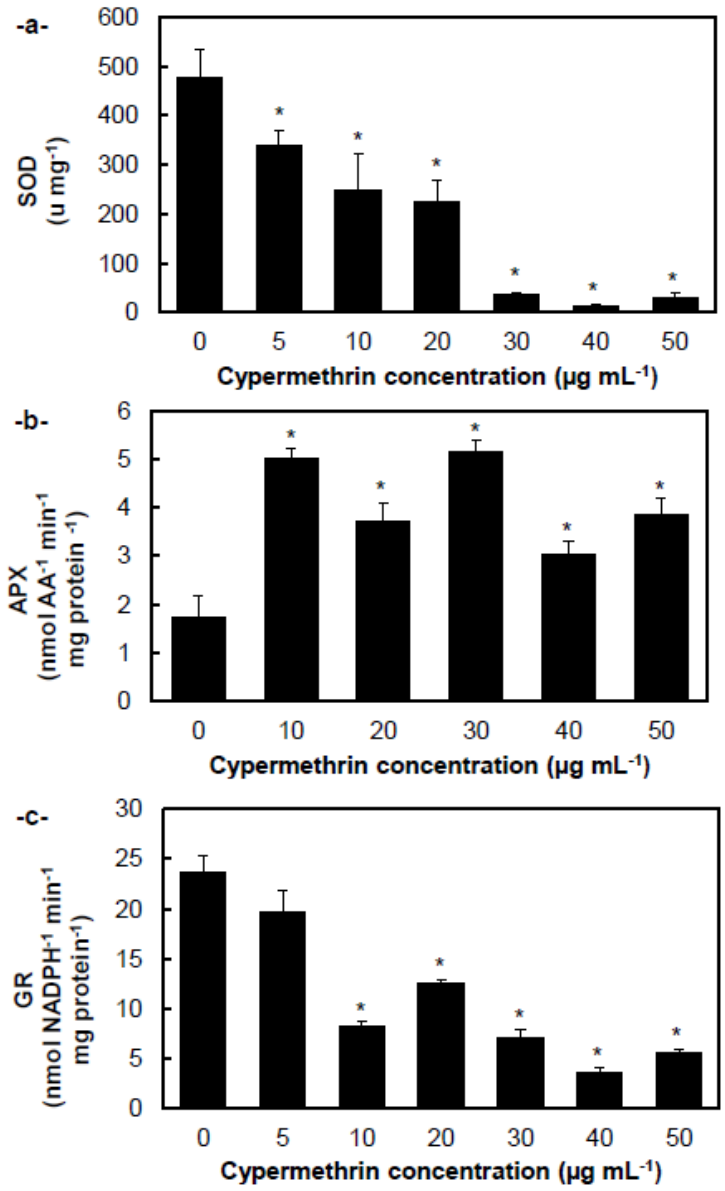

Fig. 3. Total superoxide dismutase (SOD; a), ascorbate peroxidase (APX; b) and glutathione reductase (GR; c) activities of *Arthrospira platensis* treated with 0-50 µg mL^{-1} cypermethrin. Data are the means ± SE of three replicates. *Significantly different from control, $P < 0.05$ (LSD analysis).

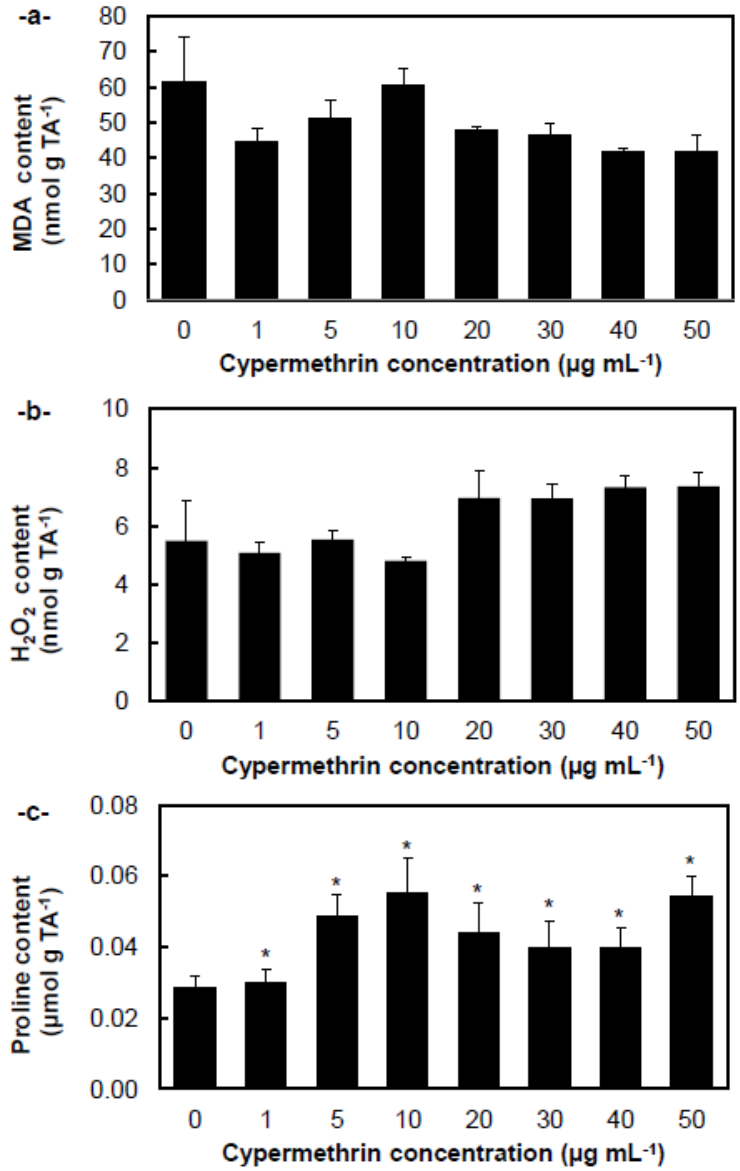

Fig. 4. Malondialdehyde (MDA; a), hydrogen peroxide (H_2O_2; b) and proline (c) contents of *Arthrospira platensis* treated with 0-50 µg mL^{-1} cypermethrin. Data are the means ± SE of three replicates. *Significantly different from control, $P < 0.05$ (LSD analysis).

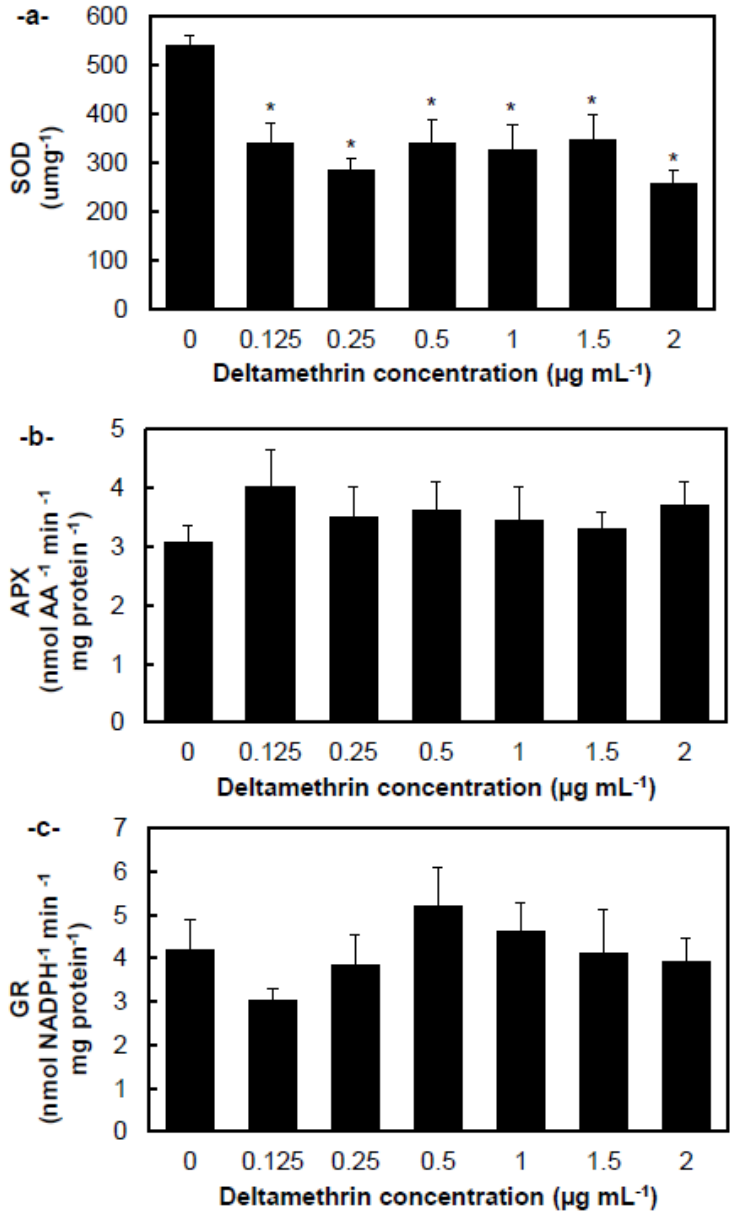

Fig. 5. Total superoxide dismutase (SOD; a), ascorbate peroxidase (APX; b) and glutathione reductase (GR; c) activities of *Arthrospira platensis* treated with 0-2 µg mL^{-1} deltamethrin. Data are the means ± SE of three replicates. *Significantly different from control, $P < 0.05$ (LSD analysis).

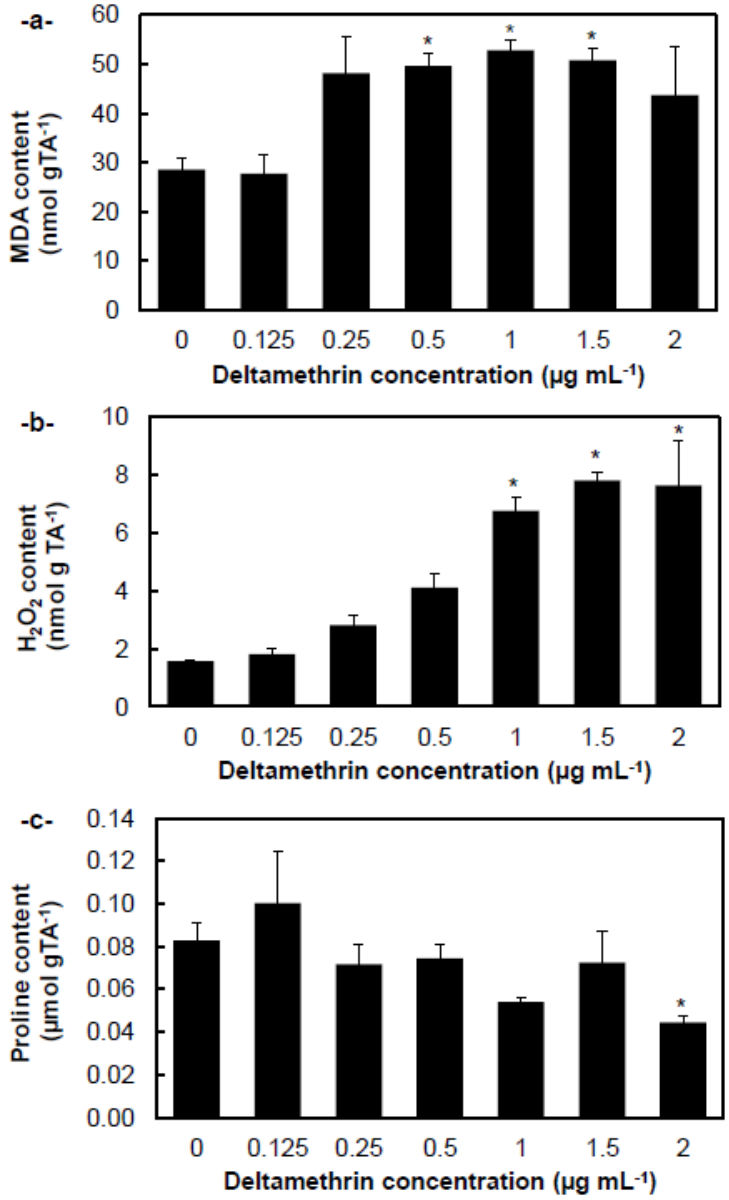

Fig. 6. Malondialdehyde (MDA; a), hydrogen peroxide (H_2O_2; b) and proline (c) contents of *Arthrospira platensis* treated with 0–2 µg mL^{-1} deltamethrin. Data are the means ± SE of three replicates. *Significantly different from control, $P < 0.05$ (LSD analysis).