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Insecticides induced biochemical changes in freshwater microalga *Chlamydomonas mexicana*

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Abstract The effect of insecticides (acephate and imidacloprid) on a freshwater microalga *Chlamydomonas mexicana* was investigated with respect to photosynthetic pigments, carbohydrate and protein contents, fatty acids composition and induction of stress indicators including proline, superoxide dismutase (SOD) and catalase (CAT). *C. mexicana* was cultivated with 1, 5, 10, 15, 20 and 25 mg L^{-1} of acephate and imidacloprid. The microalga growth increased with increasing concentrations of both insecticides up to 15 mg L^{-1}, beyond which the growth declined compared to control condition (without insecticides). *C. mexicana* cultivated with 15 mg L^{-1} of both insecticides for 12 days was used for further analysis. The accumulation of photosynthetic pigments (chlorophyll and carotenoids), carbohydrates and protein was decreased in the presence of both insecticides. Acephate and imidacloprid induced the activities of superoxide dismutase (SOD) and catalase (CAT) and increased the concentration of proline in the microalga, which play a defensive role against various environmental stresses. Fatty acid analysis revealed that the fraction of polyunsaturated fatty acids decreased on exposure to both insecticides. *C. mexicana* also promoted 25 and 21 % removal of acephate and imidacloprid, respectively. The biochemical changes in *C. mexicana* on exposure to acephate and imidacloprid indicate that the microalga undergoes an adaptive change in response to the insecticide-induced oxidative stress.

Keywords Acephate · *Chlamydomonas mexicana* · Imidacloprid · Proline · Stress enzymes · Superoxide dismutase

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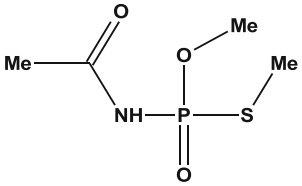
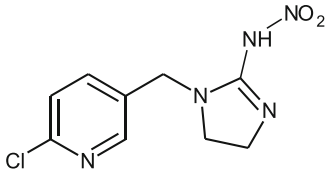
Introduction

Insecticides are widely used for crop protection in agricultural practice and have been claimed as a major factor for increased agricultural productivity in the 20th century. The unregulated and indiscriminate application of insecticides has lead to their frequent appearance in water resources. Acephate and imidacloprid (Table 1) are low poisonous insecticides and are highly soluble in water (Chuanjiang et al. 2010; Jemec et al. 2007). These insecticides have raised much concern because of their intermediates, which are more toxic than their parent compounds (Anhalt et al. 2007; Mohapatra et al. 2011). The prolonged application of acephate and imidacloprid during one crop period has lead to their accumulationin soil (Kwon and Penner 1995). The insecticides spread to water bodies due to accidental spills or spray drift, leading to local point source contamination. Insecticides are known to pose deleterious and toxic effects on humans and nontarget beneficial microorganisms such as algae and cyanobacteria (Rodger et al. 1994; Fatma et al. 2007). The toxicity of insecticides and

Table 1 Physico-chemical properties and partition coefficients of (a) acephate and (b) imidacloprid

Properties	Acephate	Imidacloprid
Chemical structure		
IUPAC name	*N*-(Methoxy-methylsulfanylphosphoryl)acetamide	*N*-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide
Molecular formula	$C_4H_{10}NO_3PS$	$C_9H_{10}ClN_5O_2$
CAS Reg. No.	30560-19-1	138261-41-3
Molecular mass	183.17 g mol^{-1}	255.661 g mol^{-1}
Water solubility	79–83.5 g 100 mL^{-1}	0.51 g L^{-1}
Henry's law constant	3.1×10^{-7} atm-m^3mol^{-1}	1.7×10^{-10} Pa-m^3mol^{-1}
Vapour pressure	1.7×10^{-6} mmHg at 24 °C	3×10^{-12} mmHg at 20 °C

their impact on aquatic environment have been rarely assessed (Gibbons et al. 2014).

Microalgae are one of the main diazotrophic components of the primary microbiota producers in paddy field and significantly contribute to building-up soil fertility (Elizabeth H. Harris 2008). Microalgae in their natural habitats are often exposed to various contaminants such as heavy metals, hexachlorobenzene, herbicides, insecticides, endocrine-disrupting chemicals, and phenol (Hirooka et al. 2005; Newsted 2004; Dosnon-Olette et al. 2010), which impose toxic effects on the microalgae. Exposure to such contaminants leads to generation of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydroxyl radicals (^-OH) and hydrogen peroxide (H_2O_2), which have high biological activity and cause eco-toxicity through oxidative damage of cellular components including lipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), DNA (Gibbons et al. 2014) and carbohydrates (Imlay et al. 1998; Vandana et al. 2001; Olga et al. 2003). Microalgae cell possesses a complex array of enzymatic antioxidant defence system, which comprises mainly enzymes superoxide dismutase (SOD) and catalase (CAT) (Srivastava et al. 2005; Wiktelius and Stenberg 2007).

Chlamydomonas mexicana, a freshwater microalga, has been reported to be tolerant and promote removal of endocrine-disrupting chemicals (EDCs) including bisphenol A (Ji et al. 2014) and atrazine (Kabra et al. 2014). Fatty acid methyl ester (FAME) profile is considered to be a useful biomarker to assess the effect of pesticides on aquatic microbial community (Littlefield-Wyer et al. 2008; Kabra et al. 2014). Considerable work has been done on cyanobacterial antioxidant system (Choudhary et al. 2007; Kumar et al. 2012a; Kumar et al. 2013), but relatively, little information is available on acephate- and imidacloprid-induced ROS generation and their detoxification in resistant algae. We have therefore investigated the biochemical changes and antioxidant enzyme system in *C. mexicana* FR751193 under acephate and imidacloprid (insecticides) exposure conditions.

Materials and methods

Chemicals, organism and culture conditions

All chemicals used in this study were of analytical grade. Acephate and imidacloprid (purity, >99.0 %) were dissolved in deionized water to prepare stock solution of 500 mg L^{-1}, which was filtered and stored at 4 °C. Working concentrations of the insecticides (1, 5, 10, 15, 20 and 25 mg L^{-1}) were obtained by diluting the stock solution with distilled water. The green microalga *C. mexicana* FR751193 was used in this study. Axenic culture of the microalga was inoculated in a 250-mL Erlenmeyer flask containing 100 mL of Bold's basal medium (BBM) at a 10 % concentration ($V_{inoculum}/V_{media}$) (Bischoff and Bold 1963). The microalgal cells were cultivated in a shaker incubator at 27 °C and 150 rpm, under 14/10-h light/dark cycle using white fluorescent light of 45–50 μmol photon m^{-2} s^{-1} for 2 weeks. The microalgal suspension in BBM was adjusted to an absorbance of 1.0 at an optical density (OD) of 680 nm as measured using a spectrophotometer (Hach DR/4000, Loveland, CO, USA) and was used as the initial inoculum for further experiments.

Experimental design

Five millilitres of microalgal suspension was inoculated in each of the 500-mL serum bottles containing 100 mL of BBM medium supplemented with acephate and imidacloprid at varying initial concentrations (1, 5, 10, 15, 20 and 25 mg L^{-1}), respectively. The cultures were incubated at 27 °C, under 14/10-h light/dark cycle using white fluorescent light of 45–50 μmol photon m^{-2} s^{-1}, and were gently shaken by the hand on alternate days. The algal culture inoculated in BBM without insecticides was used as control. The dry cell weight (DCW) was estimated after every 3-day interval up to 18 days. The alterations in photosynthetic pigments, enzyme activity and fatty acid profile of the microalga were analyzed with 15 mg L^{-1} of both insecticides at the endpoint of the experiment. The data were analyzed by one-way analysis of variance with Tukey–Kramer multiple comparison test using Graphpad Prism 5 (Motulsky 2007), and the results were expressed as means±standard deviation (SEM) of triplicate.

Growth measurement

The growth of *C. mexicana* was monitored by changes in the OD_{680} absorbance. The OD_{680} values were converted to DCW based on a relationship between OD_{680} and DCW, which was obtained after multiple data analysis and was calculated by Eq. 1 (Kabra et al. 2014).

$$\text{Dry cell weight}\left(gL^{-1}\right) = 0.3218 \times OD_{680} - 0.0139 \left(R^2 = 0.9948\right) \quad (1)$$

Biochemical analysis

The microalgae cultures subjected to 15 mg L^{-1} of acephate and imidacloprid for 12 days were harvested by centrifugation (5000 rpm for 10 min) and subjected for determination of chlorophyll, carotenoid, carbohydrate, protein, proline, SOD and CAT contents and FAME profile. *C. mexicana* cells without insecticides in the medium served as a control.

Estimation of photosynthetic pigments, carbohydrate and protein contents

Total chlorophyll was extracted in 80 % methanol and measured spectrophotometrically. The absorbance of the supernatant at λ=665 and 652-nm wavelengths was determined with a Hach DR/4000 UV-visible spectrophotometer (Porra et al. 1989). Carotenoid was extracted in 85 % methanol and measured spectrophotometrically at 480 nm (Hellebust and Craige 1978). The carbohydrate and protein estimation was performed using phenol sulphuric acid and Lowry methods, respectively (DuBois et al. 1956; Lowry et al. 1951).

Estimation of proline, superoxide dismutase and catalase

For estimation of proline, the algal cells were suspended in 10 mL of 3 % sulphosalicylic acid for 2 min and centrifuged at 7000 rpm for 10 min to remove the cell debris. Two millilitres of ninhydrin was added to 2 mL of supernatant, followed by addition of 2 mL glacial acetic acid and incubation at boiling temperature for 1 h. The mixture was extracted with toluene, and proline was quantified spectrophotometrically at 520 nm from the organic phase (Bates et al. 1973). To estimate superoxide dismutase, algal biomass (50 mg) was homogenized in 2-mL 0.1 M phosphate buffer (pH 7.5). Supernatant obtained after centrifugation of the homogenate at 15,000 rpm at 4 °C was used for the enzyme assay. SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT), using a reaction mixture consisting of 1 M Na_2CO_3, 200 mM methionine, 2.25 mM NBT, 3 mM EDTA, 60 μM riboflavin and 0.1 M phosphate buffer (pH 7.8). Absorbance was read at 560 nm (Dhindsa et al. 1981). For catalase estimation, 50 mg of algal biomass was collected and homogenized with 2 mL of extraction buffer (0.5 M phosphate buffer, pH 7.5). The homogenate was centrifuged at 12,000 rpm for 20 min, and the supernatant (enzyme extract) was used for catalase assay. To 100 μL of enzyme extract, 1.6 mL phosphate buffer, 0.2-mL 0.3 % H_2O_2 and 3 mM EDTA were added in a test tube, and the reaction was allowed to run for 3 min. One unit of enzyme is the amount necessary to decompose 1 μL of H_2O_2 per minute at 25 °C. The absorbance of the supernatant was observed at 240 nm against blank (Aebi 1984).

Analysis of fatty acids

The composition and content of fatty acid were determined using a modified direct transesterification method (Lepage and Roy 1984). The fatty acid methyl esters were analyzed by gas chromatography with a flame ionization detector and a HP-INNO Wax capillary column (Agilent Technologies, USA). Helium was used as a carrier gas at 2.2 mL min^{-1}. The injection volume and split ratio were 2 μL and 45:1, respectively. Injector and detector temperatures were set at 250 and 275 °C, respectively. Mix RM3, Mix RM5, GLC50 and GLC70 (Supelco, USA) were used as standards.

Determination of acephate and imidacloprid with online UPLC-orbitrap mass spectrometry

Microalga cell-free supernatant was extracted with centrifugation at 5000 rpm for 15 min. The supernatant was used for determination of residual acephate and imidacloprid in the medium. All the samples were stored at 4 °C in the dark prior to measurement. Prior to UPLC analysis, acephate and imidacloprid in water samples were preconcentrated by

performing online solid phase extraction with an EQUAN MAXTM system (Thermo Fisher Scientific, CA, USA). This method is based on column switching techniques and consists of a trap column and an analytical column. The trap and analytical columns used were the Hypersil Gold aQ (20 mm, 12-μm particle size) and Hypersil Gold (50 mm, 1.9-μm particle size), respectively, from Thermo Fisher Scientific. The sample delivery system consists of a CTC PAL auto sampler manufactured by CTC analytics (Zwingen, Switzerland), a six-port switching valve and a quaternary load pump. High-resolution Orbitrap MS was performed on the Exactive model with an electrospray ionization source and 50,000 resolving power (Thermo Fisher Scientific, CA, USA).

Result and discussion

Growth behaviour

Different concentrations (1, 5, 10, 15, 20 and 25 mg L^{-1}) of acephate and imidacloprid showed varying effect on the growth of *C. mexicana* (Fig. 1a, b). The DCW of *C. mexicana* increased with increasing concentration of both insecticides up to 15 mg L^{-1}, beyond which the growth declined. *C. mexicana* showed no growth inhibition on exposure to bisphenol A up to 25 mg L^{-1}, while its growth was inhibited by 36 % on exposure to 100 μg L^{-1} of atrazine. This can be attributed to the differential permeability of the contaminants across the cell membrane (Fatma et al. 2007), leading to differential sensitivity of *C. mexicana* towards different contaminants. At 15 mg L^{-1}, imidacloprid and acephate increased the growth of *C. mexicana* up to 0.19 and 0.16 g DCW L^{-1}, respectively, compared to control (0.08 g DCW L^{-1}) after 12 days, which might be due to consumption of the insecticides as a source of carbon energy (Felsot et al. 1989; Cycon et al. 2009). Ingram et al. (2005) also reported that imidacloprid had no toxic effect on *P. vulgaris* at lower concentration (0.70 g L^{-1}). The DCW of the microalga declined at 25 mg L^{-1} of both insecticides, which might be due to toxicity at high concentrations. Similar results have also been reported for photosynthetic cyanobacteria treated with endosulfan (Satish and Tiwari 2000; Kumar et al. 2008), *Anabaena* sp. with benthiocarb and butachlor (Zarger and Dar 1990) and *Anabaena cylindrica* and *Anabaena variabilis* with propanil (Wright et al. 1997).

Biochemical analysis

Photosynthetic pigments

The chlorophyll *a* and carotenoid contents of *C. mexicana* exposed to 15 mg L^{-1} of acephate and imidacloprid have been illustrated in Fig. 2. The chlorophyll (38.75 and 28.94 mg g^{-1}) and carotenoid (13.75 and 11.58 mg g^{-1}) contents were decreased on exposure to acephate and imidacloprid, respectively (Fig. 2a, b). Exposure of different microalgal species to pesticides including atrazine and endosulfan has been reported to decrease the accumulation of chlorophyll (Kumar et al. 2008; Kabra et al. 2014). The chlorophyll and carotenoid contents of cyanobacterial species were also decreased on exposure to pesticides including chlorpyrifos, endosulfan and tebuconazole. Polyunsaturated fatty acids in chloroplast membrane are highly susceptible to peroxidation leading to inhibition of chlorophyll synthesis (Kumar et al. 2012b; Kumar et al. 2014). Thus, the pesticide-induced stress imposes an inhibitory effect on the pigment synthesis.

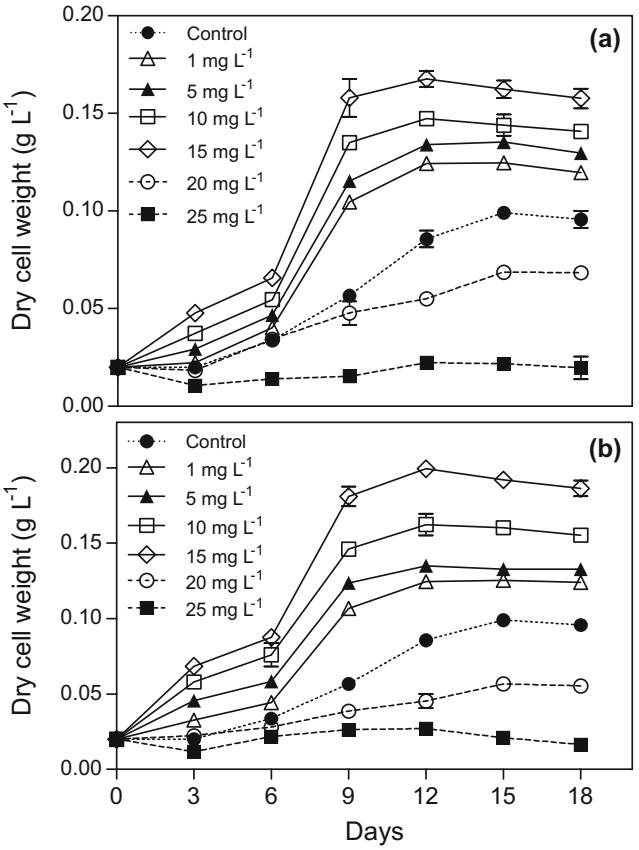


Fig. 1 Effect of **a** acephate and **b** imidacloprid, on the dery cell weight dry cell weight (DCW) of *C. mexicana* at 15 mg L^{-1} for 18 days. Values are a mean of three experiments±SEM

Carbohydrate

The carbohydrate accumulation in microalgae is strongly influenced by external environmental conditions. The carbohydrate content of *C. mexicana* exposed to both insecticides was higher than the control (Fig. 2d). The carbohydrate content increased from 81 mg g^{-1} (control) to 118 and 106 mg g^{-1} with 15 mg L^{-1} of acephate and imidacloprid, respectively. Similar observations have been reported for *Nostoc kihlmani*,

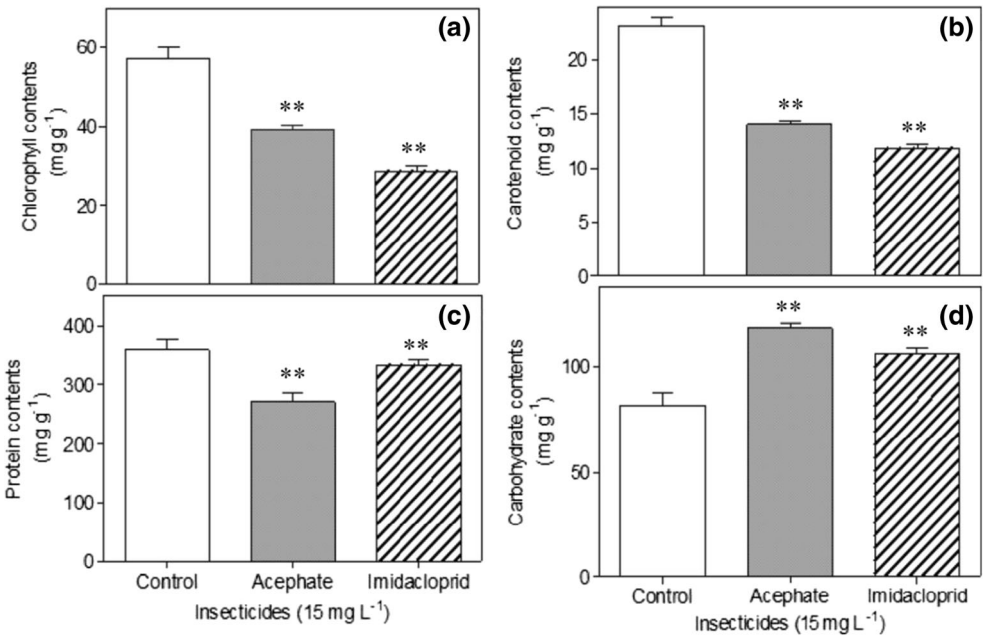


Fig. 2 Effect of acephate and imidacloprid on **a** chlorophyll, **b** carotenoid, **c** protein and **d** carbohydrate contents at 15 mg L^{-1} after 12th day of treatment. Values are a mean of three experiments±SEM. Significantly different from control (0 h) at $^{**}P<0.001$ by one-way ANOVA with Tukey–Kramer comparison test

Nostoc muscorum, *Anabaena variabilis*, *Anabaena oscillariodes* and *Scenedesmus quadricauda*, where exposure to the insecticide thiobencarb increased the contents of reducing sugar, sucrose, polysaccharides and total sugars (Kumar et al. 2008; El-Salam Issa et al. 2013). Increasing atrazine concentration (25–100 μg L^{-1}) increased the carbohydrate content of *C. mexicana* up to 47–52 % (Kabra et al. 2014). The increase in carbohydrate content on exposure to insecticides might be due to diversion of the energy production towards storage of intracellular carbohydrates. Higher chlorophyll synthesis in *C. mexicana* on exposure to both insecticides will enhance the photosynthetic activity, resulting in higher carbohydrate accumulation.

Protein and proline

The total protein content of *C. mexicana* was decreased from 358 mg g^{-1} (control) to 271 and 334 mg g^{-1} on exposure to 15 mg L^{-1} of acephate and imidacloprid, respectively (Fig. 2c). Similar observations have been reported for *Anabaena* sp. treated with endosulfan (0.5–2 μg mL^{-1}) (Kumar et al. 2008; Babu et al. 2001). The decrease in protein content may be due to toxicity of the insecticides leading to generation of reactive oxygen species (ROS) (Leitao et al. 2003). Studies related to analysis of proline synthesis in microalgal cells on exposure to insecticides are scarce. Free proline accumulation has been considered as an important biomarker of tolerance capacity against various stresses in plants, bacteria, protozoa, cyanobacteria and marine invertebrates. Proline functions in crucial cellular detoxification mechanism and scavenging of ROS (Kumar et al. 2014), thereby reducing the damage due to oxidative stress. *C. mexicana* cells in this study responded by increasing the proline content under acephate and imidacloprid tested conditions. The amount of proline in acephate and imidacloprid (15 mg L^{-1}) exposed *C. mexicana* cells was 107.08±0.09 and 98.03±0.04 mg g^{-1} of biomass, respectively, compared to control (66.66±0.11 mg g^{-1}) (Fig. 3c). Increased amount of proline was observed in cyanobacterial species exposed to bentazon (herbicide) (Fatma et al. 2007; Galhano et al. 2011b). Recent studies have also reported high cellular proline accumulation in many cyanobacterial species exposed to pesticides (Galhano et al. 2011a, b). The increase in proline content can be an adaptive response against lipid peroxidation under stress conditions (Fatma et al. 2007; Kumar et al. 2014). Proline reduces the generation of free radicals under stress conditions (Alia and Saradhi 1993).

Antioxidants (SOD and CAT)

Superoxide dismutase activity (SOD) Figure 3a represents the superoxide dismutase activity in *C. mexicana* on exposure to acephate and imidacloprid. Activity of SOD in microalga exposed to 15 mg L^{-1} of acephate and imidacloprid was 145.14±0.06 and 134.16±0.04 unit mg^{-1} protein, respectively. The SOD activity was higher under insecticide exposure conditions than the control (121.04±0.07 unit mg^{-1} protein) (without insecticides). Organic pollutants such as herbicides and insecticides become accumulated and biotransformed in the microorganisms that further leads to generation of reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals and hydrogen peroxide (Vandana et al. 2001). To prevent the damage caused by superoxide radicals, the cell system increases the activity of superoxide dismutase, which

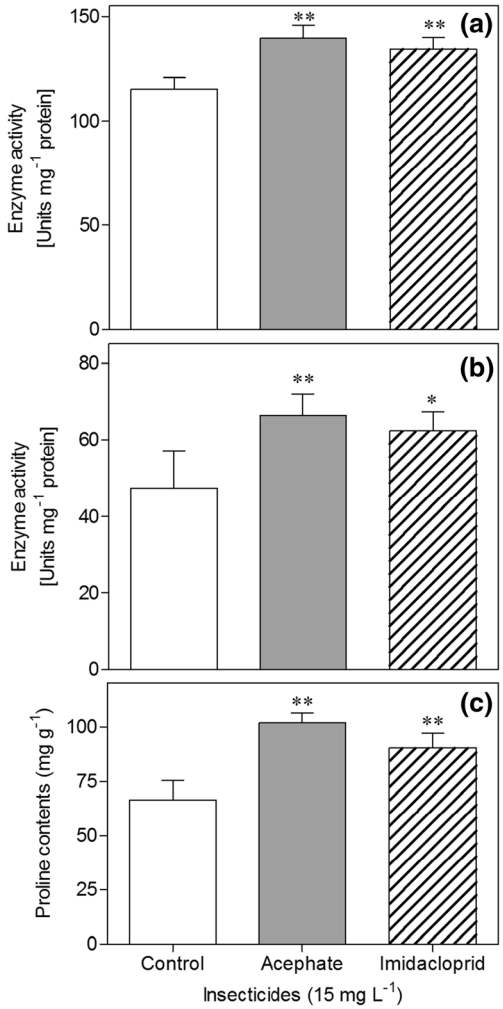


Fig. 3 Effect of acephate and imidacloprid on **a** SOD and **b** CAT activities, and **c** proline accumulation, in *C. mexicana* at 15 mg L^{-1} after 12th day of treatment. Values are a mean of three experiments± SEM. Significantly different from control (0 h) at $^{**}P<0.001$ and $^{*}P<0.01$ by one-way ANOVA with Tukey–Kramer comparison test

catalyzes the neutralization of superoxide anion to hydrogen peroxide. The first line of defence mechanism against the generation of toxic oxygen species is the induction of SOD (Janknegt et al. 2007). Antioxidants are generated in the cell especially under high environmental stress conditions (Okamoto et al. 2001). The SOD activity has been reported to increase in *Chroococcus turgidus*, *Nostoc muscorum*, *Anabaena variabilis* and *Aulosira fertilissima*on exposure to endosulfon (10 mg L^{-1}) and chlorpyrifos (6 mg L^{-1}) (Galhano et al. 2011b; Kumar et al. 2014).

Catalase activity (CAT) Figure 3b represents the catalase activity in *C. mexicana* under control and acephate and imidacloprid exposure conditions. The catalase activity in control, acephate and imidacloprid treated cells was 58.07 ±0.05, 68.11±0.08 and 63.03±0.05 unit mg^{-1} protein, respectively. Increased production of hydrogen peroxide under pesticide stress condition can be due to inactivation of water splitting complex. Catalase decomposes the hydrogen peroxide into water and oxygen. Increase in catalase activity was observed in endosulfan and bentazon treated *Plectonema boryanum* and *Nostoc muscorum*, respectively (Prasad et al. 2005; Galhano et al. 2011b). A high antioxidant concentration in algal and cyanobacterial cells has been related to increased tolerance against different kinds of environmental stresses (Okamoto et al. 2001; Kumar et al. 2012a). The ability of microorganisms (bacteria, cyanobacteria and algae) to overcome oxidative stress can be attributed to the levels and types of antioxidative enzymes that they possess (Lin et al. 2009; Kumar et al. 2012a; Kumar et al. 2013). The combined action of SOD and CAT is critical for mitigating the effects of oxidative stress. In this study, the presence of antioxidant enzymes under control conditions indicates the occurrence of ROS, which are involved in signal transduction mechanisms. Under insecticide stress conditions, the level of ROS should be high, leading to increased production of antioxidant enzymes for protecting the cells against the oxidative damage caused by the ROS-generated stress.

Fatty acid methyl ester (FAME)

The fatty acid profile with respect to composition of saturated and unsaturated fatty acids of *C. mexicana* under control and insecticide exposure conditions is shown in Table 2. Exposure of *C. mexicana* to acephate and imidacloprid induced significant alterations in the fatty acid profile. The total saturated fatty acids were increased, while the unsaturated fatty acids were decreased in the presence of both insecticides. Fatty acid profile is considered as a useful biomarker to assess the effect of pesticides on aquatic microbial communities. The cyanobacterium, *Nostoc muscorum*, showed an increase in level of saturated fatty acids and a decrease in level of unsaturated fatty acids on exposure to pesticides bentazon and molinate (Galhano et al. 2011a, b). Exposure to pesticide atrazine also increased the fatty acid saturation in *C. mexicana* (Kabra et al. 2014). Cell membranes, which are structurally made up of large amounts of polyunsaturated fatty acids, are highly susceptible to the pesticide-induced oxidative stress, leading to inevitable changes in membrane fluidity and permeability (Singh et al. 2002; Galhano et al. 2011a). Thus, lowering the degree of fatty acid unsaturation might be an adaptive mechanisms by the algal cells against the insecticide-induced oxidative stress.

Removal of acephate and imidacloprid by *C. mexicana*

Acephate and imidacloprid in the medium were quantified using UPLC after 12 days of incubation period (Fig. 4). *C. mexicana* promoted the removal of acephate and

Table 2 Fatty acid profile of *C. mexicana* exposed to acephate and imidacloprid at 15 mg L^{-1} for 12 days

Fatty acids	Normal[a] % (*w*/*w*)	Acephate treated[b] % (*w*/*w*)	Imidacloprid treated[c] % (*w*/*w*)
16:0	1 8.45±0.04	22.40±0.01	21.94±0.04
18:0	0.57±0.06	0.34±0.05	0.28±0.06
Total saturated fatty acids	**19.02±0.05**	**22.74±0.03**	**22.22±0.05**
C16:1	2.26±0.02	1.98±0.03	1.32±0.12
C18:1n9c	3.73±0.12	2.19±0.05	1.44±0.09
C18:2n6c	19.49±0.09	19.64±0.08	19.03±0.04
C18:3n3	20.74±0.04	16.59±0.05	21.69±0.12
Total unsaturated fatty acids	**45.22±0.06**	**40.4±0.05**	**43.48±0.09**
Others	34.76±0.03	36.86±0.05	34.3±0.11

[a] Culture grown on BBM medium

[b] Culture grown on BBM medium with 15 mg L^{-1} acephate

[c] Culture grown on BBM medium with 15 mg L^{-1} imidacloprid

Data represent the mean±standard deviation of triplicate

imidacloprid by 25 and 21 %, respectively. The removal of insecticides by the microalga was low, which might be due to high concentration of insecticides and low microalgal population. Researchers have reported that with a lower inoculum level, the microorganisms were not able to promote the degradation of organic contaminants (Singh et al. 2006; Ramu and Seetharaman 2014). Increased *C. mexicana* growth in the presence of acephate and imidacloprid (15 mg L^{-1}) might be due to utilization of the insecticides as a source of carbon energy (Cycon et al. 2009). The responses of algae to pesticides are little or no effect at low doses, ranging up to growth inhibition at high doses. Induction of biochemical changes in *Chlorella* sp. was slight with 10 mg L^{-1} methyl parathion, moderate with 20 mg L^{-1} and severe with 30 mg L^{-1} (Saroja and Bose 1982). Our results proved that *C. mexicana* was able to tolerate high concentration of acephate and imidacloprid, and further research should be conducted on the biochemical and genetic aspects of insecticide degradation by *C. mexicana*.

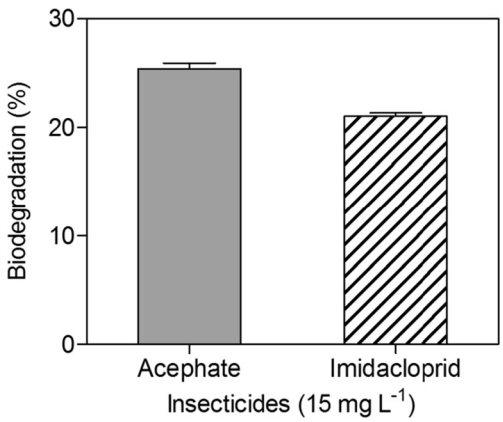


Fig. 4 Removal of acephate and imidacloprid by *C. mexicana* at 15 mg L^{-1} after 12th day of treatment

Conclusions

Insecticides, acephate and imidacloprid had no negative effect on the growth of *C. mexicana* up to 15 mg L^{-1}, beyond which the growth was decreased below control. Alteration in the contents of carbohydrate and protein indicates the insecticide stress-induced biochemical changes in the microalga. Increase in the proline content and activity of antioxidant enzymes including SOD and CAT was evident in *C. mexicana* cells exposed to acephate and imidacloprid, indicating the defensive response of the microalga against the insecticide-induced stress. Increased activity of cellular antioxidants and decreased levels of cellular unsaturated fatty acid content indicate the adaptive defence mechanisms of *C. mexicana* against acephate- and imidacloprid-induced stress.

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