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EFFECT OF PYRETHROID ON GROWTH AND PROTEIN CONTENT OF TWO UNICELLULAR CYANOBACTERIA (BLUE GREEN ALGAE)

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Abstract

Two unicellular strains of cyanobacteria *Chroococcidiopsis* sp. and *Microcystis* sp. isolated from marine environment were mass cultured and their interaction with pyrethroid pesticides was studied. Pyrethroid pesticides namely cypermethrin and deltamethrin are used widely as agricultural insecticides and have been identified from many environments including soil and water. It is also reported from agricultural runoff and coastal waters of Pakistan. In the current study both strains were exposed to different concentration of pesticides in laboratory conditions. Tolerance limit of these cyanobacteria was determined in different concentrations ranging from 5-500 µg/mL by studying photosynthetic pigments (chlorophyll *a* and carotenoids), growth rate *via* dry weight and total proteins. Results showed that these cyanobacterial isolates tolerate cypermethrin up to 100 µg/mL. It was also observed that the presence of pesticides in the media enhanced protein content as compared to control. However, deltamethrin has showed deleterious effects on growth and photosynthetic pigments. Protein content was found decreasing in deltamethrin treated cultures.

Key words: *Chroococcidiopsis* sp., *Microcystis* sp., Cypermethrin, Deltamethrin, Toxicity, Protein content.

Introduction

Pyrethroid pesticides are mostly used in agricultural fields to control pests due to their non-persistent nature in the environment (Kumar *et al*., 2010). Pesticides affect non-targeted organisms including cyanobacteria. The intensity of pyrethroid effects on different organisms relies on exposure time and wash-away concentration (Vijverberg & Bercken, 1990; Patra, 2000). Many studies demonstrated the lethal effects of different pesticides on cyanobacteria (blue green algae) also known as Cyanophyta (Vijverberg & Bercken, 1990; Galhano, 2008, Shoaib *et al.,* 2012; Singh *et al.,* 2016). High acute toxicity of these pesticides affects some non-target species, which has been proved in many laboratory tests (Mohapatra *et al.,* 2003; Phyu *et al.,* 2004; Sial *et al*., 2009). Pyrethroids are mostly preferred due to their degradation into less toxic or non-toxic products (Golow & Godzi, 1994). Several pesticides such as 2-methoxy-ethyl mercury chloride, manganese ethylene bisdithiocarbamate, endosulfan and phosphorodithioate are reported to inhibit the growth, biochemical content, and enzymatic activity of cyanobacteria (Debnath *et al.,* 2012). It is reported that pesticides affect microalgae by inhibiting their enzymatic and photosynthetic activity, altering the permeability of cell membrane and interfering with the production of nucleic acid and proteins (Lal & Saxena, 1980).

Present study is first of its kind from coastal water of Pakistan and aimed to explicate the interaction of cypermethrin and deltamethrin on two unicellular cyanobacterial strains *Chroococcidiopsis* sp. (MB.0023) and *Microcystis* sp. (MB. 0024). These strains were exposed to various concentrations of the pyrethroids and changes in photosynthetic pigments, growth rate and protein were also observed. No research work has been reported on the effects of interaction of pyrethroid on cyanobacterial isolates from the coastal waters of Pakistan.

Materials and Methods

Isolation, purification and mass culture: Cyanobacterial strains were isolated from Sandspit back waters, Karachi between Manora and Hawksbay (24°49'N and 66°56'E). Isolation and purification of these strains was done by using plating method as described by Stanier *et al.,* 1971. Cyanobacteria were grown in ASN III media (Rippka *et al.,* 1979) under photoautotrophic conditions. The batch cultures were kept at 32±2°C. Culture vessels were illuminated with intensity of 25 µmol/m^2/s with light/dark cycle (14/10 h). Cyanobacterial strains were enumerated at x100 magnification (Olympus IX-51, Japan) and identified following Komárek & Hauer, (2013). The voucher specimens were submitted in the CEMB under the code MB.0023 for *Chroococcidiopsis* sp. and MB.0024 for *Microcystis* sp.

Estimation of growth measurement: The growth of cyanobacteria was assessed as dried biomass at an interval of 5 days up to 30 days. The biomass was oven dried at 60°C for overnight and expressed as mg/mL (Yamamoto & Tsukada, 2009).

Estimation of chlorophyll *a* and carotenoids: Aliquots of 10 mL media were taken from each flask and filtered through glass fibre filters (GF/F 0.7 µm) Whatman®. Filtrate was washed thoroughly with distilled water. Equal volume of acetone was added in cells and incubated at 4°C in dark for 48 h. Samples were centrifuged at 3000 xg for 20 min and filtrate was analysed for chlorophyll *a* and carotenoids using spectrophotometer by using equation proposed by Strickland & Parsons, (1972).

Estimation of protein: Total protein was estimated by using Folin-phenol reagent method outlined by Lowry *et al.,* 1951. In a test tube 100 mg of cyanobacterial biomass was taken and 1 mL of NaOH (1 N) was added. The test tube was kept in a boiling water bath for 10 minutes. 5

mL of reagent A (prepared by adding 1 mL of Na-K tartarate solution and 0.5% $CuSO_4$ in 50 mL of 2% Na_2CO_3 solution) was added and then incubated at room temperature for about 10 minutes. 0.2 mL of reagent B (Folin reagent) was also added, properly mixed the content and again incubated for 30 minutes at room temperature. The absorbance of the supernatant was read at 660, 700 and 750 nm against blank. Protein content was calculated using trivial formula:

Protein (% w/w) in sample = [(C x V x D) / m] x 100

where, c = mg / L of protein in sample from calibration curve, V = L of solvents used for extraction, D = dilution factor, if any and m = mg of biomass taken.

Results

Effect of pyrethroids on chlorophyll *a* and carotenoids of *Chroococcidiopsis* sp. and *Microcystis* sp.: Two unicellular strains of cyanobacteria were isolated from Karachi coast, *Chroococcidiopsis* sp. and *Microcystis* sp. (Fig. 1). Different concentrations of pyreyhroids (5, 20, 50, 100 and 500 µg/mL) showed significant effects on the chlorophyll *a* and carotenoids of cyanobacterial cultures of *Chroococcidiopsis* sp. and *Microcystis* sp. (Table 1). Growth of cyanobacteria was observed to be increased in both strains at low concentration of pesticides (5-50 µg/mL) reaching the peak on the 30th day (Fig. 2). Initially the growth was found to be decreased but after day 15 both of these strains showed tolerance against pesticide which is proved by their increased growth. However, at higher concentrations (100 and 500 µg/mL) complete inhibition photosynthetic pigments was observed.

Deltamethrin on the other hand showed lethal effects on photosynthetic pigments of both species (Fig. 3). In case of *Microcystis* sp. all concentrations of deltamethrin were found to be inhibitory. However, both pigments were found to be increased in *Chroococcidiopsis* sp. at lower concentrations (5-20 µg/mL).

Effect of pyrethroids on growth rate of *Chroococcidiopsis* sp. and *Microcystis* sp.: Data present in Table 2 indicated the effect of different concentrations (5-500 µg/mL) of pyrethroids on the growth rate of cyanobacterial cultures of *Chroococcidiopsis* sp. and *Microcystis* sp. Addition of different concentrations (5-500 µg/mL) of cypermethrin showed that the growth rate of the both strains were inhibited. Growth rate of cyanobacteria was observed to be increasing in test strains at low concentration of pesticides (5-50 µg/mL) but it was found to be lesser in comparison to controlled cultures. However, at higher concentrations (100 and 500 µg/mL) the retarded growth rate was observed. On the other hand deltamethrin showed inhibitory effects on both strains. Growth rate was found to be decreasing with increasing concentrations of pesticide.

Effect of pyrethroids on total proteins of *Chroococcidiopsis* sp. and *Microcystis* sp.: Results of effects of pyrethroid pesticides on the total protein content of cyanobacterial strains are presented in Table 3. Total proteins were found to be increased in cypermethrin treated strain of *Chroococcidiopsis* sp. by 0.8% and 0.5% in *Microcystis* sp. However, in case of treatment with deltamethrin total protein content found to be decreased by 4.4% in *Chroococcidiopsis* sp. and 1.8% in *Microcystis* sp. calculated by obtaining the difference as follows:

% Difference in proteins content = ((A – B) / A) x 100

where, A = Protein content in control and B = protein content in test.

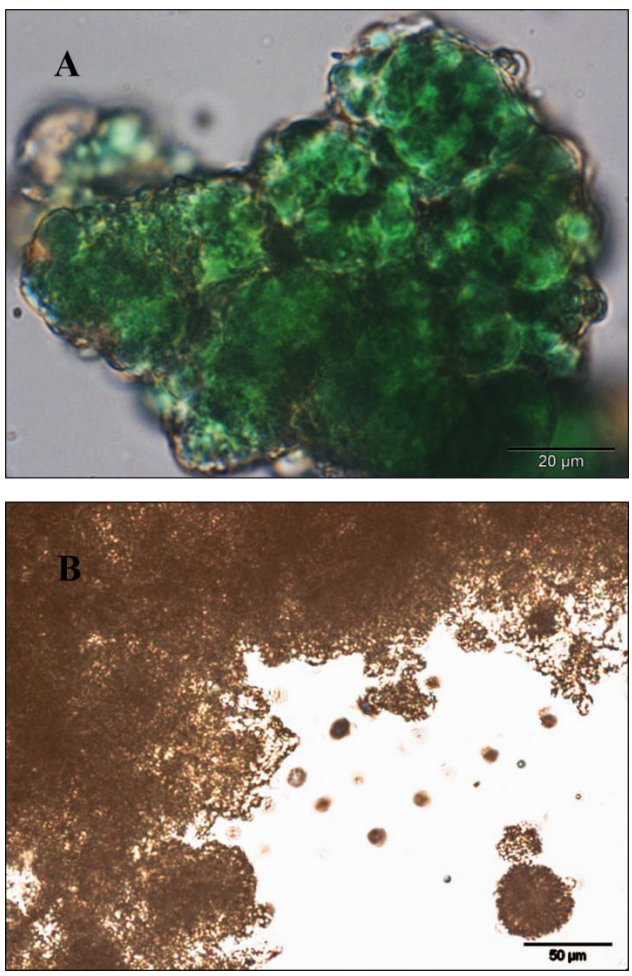

Fig. 1. Micrographs (A) *Chroococcidiopsis* sp. (B) *Microcystis* sp.

Discussion

Addition of different concentrations (5-500 µg/mL) showed direct relation of pyrethroid toxicity with its concentrations. Chlorophyll *a* and carotenoids in both strains showed varying degree of inhibition from their first day of exposure to day 30. Cypermethrin, at lower concentrations, was found to be either slightly stimulatory to both species. Treatment with cypermethrin resulted in an increase in chlorophyll *a* and carotenoid content. Higher concentrations of cypermethrin were found to be deleterious against both strains of cyanobacteria. Selvakumar *et al*., (2000) reported that *Anabaena* sp. has ability to tolerate varying concentrations of butachlor pesticide without any significant reduction in the chlorophyll *a* content.

However, deltamethrin has inhibitory effects on photosynthetic pigments of both species. An inverse relationship has been found between deltamethrin concentrations and algal strains.

The results of deltamethrin treatment are in accordance with findings of other studies (Lutnicka *et al.,* 2014; Chen *et al.,* 2016). Mohapatra & Schiewer, (2000) have reported that treatment of unicellular cyanobacteria, *Synechocystis* with dimethoate is responsible for change in fluorescent behaviour and pigments content by the interaction with cellular membrane. It was also reported that pesticides are responsible for growth inhibition in cyanobacteria as chlorophyll *a* content was reduced after treatment (Leboulanger *et al.,* 2009).

Kapoor & Arora, (1996) revealed that the synthesis of essential cell proteins including structural or growth proteins is interrupted due to enzymes inhibition. Mostafa & Helling, (2002) also suggested that in *Chlorella kesslerei*, synthesis of chlorophyll *a* and carotenoid contents decreased when microalgae were exposed to Lindane. Abdel-Raouf & El-Shafey, (2009) studied the inhibition of growth and nitrogen fixation in cyanobacteria when treated with endosulfan. These results are also in agreement with findings of Singh *et al*., (1983). They described the growth inhibition and reduction in heterocyst formation of *Nostoc linckia* when treated with thiocarbamate, a benthiocarb. Shabana-Effat, (1991) also reported the similar results that growth rate and dry mass of *Anabaena oryzae* and *Aulosira fertilssima* were decreased when treated with parathion.

The total protein content in the test strains in current study was found higher when compared with control in cypermethrin treated experiments. Mishra *et al.,* (2018) suggested that total protein content slightly induced in malathion treatments. Kumar *et al.,* (2008) observed the similar result with lower concentrations of endosulfan on *Aulosira fertilissima.* This finding suggested that lower concentrations of pesticides stimulate the production of stress induced proteins. Growth, survival rate and nitrogen fixing ability of *Anabaena* sp. and *Westiellopsis prolifica* inhibited by the exposure of carbaryl pesticide above 50 µg/mL (Adhikary *et al.*, 1984).

In case of deltamethrin decrease in protein content was observed in both strains of *Chroococcidiopsis* sp. and *Microcystis* sp. The protein content including phycobiliproteins levels decline in microflora as reported by Lal & Saxena, (1980) while studying the interaction with butachlor herbicide.

In stressed condition pattern of protein synthesis in cyanobacteria cells usually modified which results in reduced production of normal cell proteins and in some cases enhanced the synthesis of special stressed induced proteins. The reduction in protein content attributed to the presence of pesticides in environment beyond tolerance limit, higher protease activity, slow growth rate, decrease in absorption of carbon and nitrogen (Kumar et *al*., 2008). Ross *et al*., (2006) proposed that paraquat toxicity has resulted in induced synthesis and release of microcystin in water.

Table 1. Analysis of Variance (ANOVA) indicating the effects of different concentrations of cypermethrin and deltamethrin on chlorophyll *a* and carotenoids of cyanobacterial strains.

Pyrethroids	Strains	Chlorophyll *a*	Carotenoids
Cypermethrin	*Chroococcidiopsis* sp.	47***	26***
	Microcystis sp.	53***	21***
Deltamethrin	*Chroococcidiopsis* sp.	32.4***	53***
	Microcystis sp.	28***	35***

Numbers are *F*-values. Where, *** = $p<0.001$

Table 2. Effect of pyrethroids on growth rate of *Chrooco7ccidiopsis* sp. and *Microcystis* sp.

Pesticide concentration (µg/mL)	Growth rate/day			
	Cypermethrin		Deltamethrin	
	***Chroococcidiopsis* sp.**	***Microcystis* sp.**	***Chroococcidiopsis* sp.**	***Microcystis* sp.**
Control	0.59	0.42	0.59	0.42
5	0.57	0.40	0.57	0.42
20	0.40	0.38	0.42	0.21
50	0.38	0.31	0.32	0.12
100	0.23	0.22	0.12	0.04
500	0.08	0.08	0.06	0.02

Table 3. Effect of pyrethroids on Total Proteins (%) of *Chroococcidiopsis* sp. and *Microcystis* sp.

Treatment	Species	
	***Chroococcidiopsis* sp.**	***Microcystis* sp.**
Control	6.0 ± 0.2	3.8 ± 0.1
Cypermethrin	6.8 ± 0.1	4.2 ± 0.1
Deltamethrin	1.6 ± 0.1	2.0 ± 0.1

Conclusion

The present study suggests that two cyanobacterial strains *Chroococcidiopsis* sp. and *Microcystis* sp. are able to tolerate lower concentrations of cypermethrin and deltamethrin. The deltamethrin was found to be more deleterious as compare to cypermethrin. Growth rate of cyanobacterial strains reduced in cultures exposed to pyrethroid. A significant change was observed in pyrethroid treated cyanobacteria.

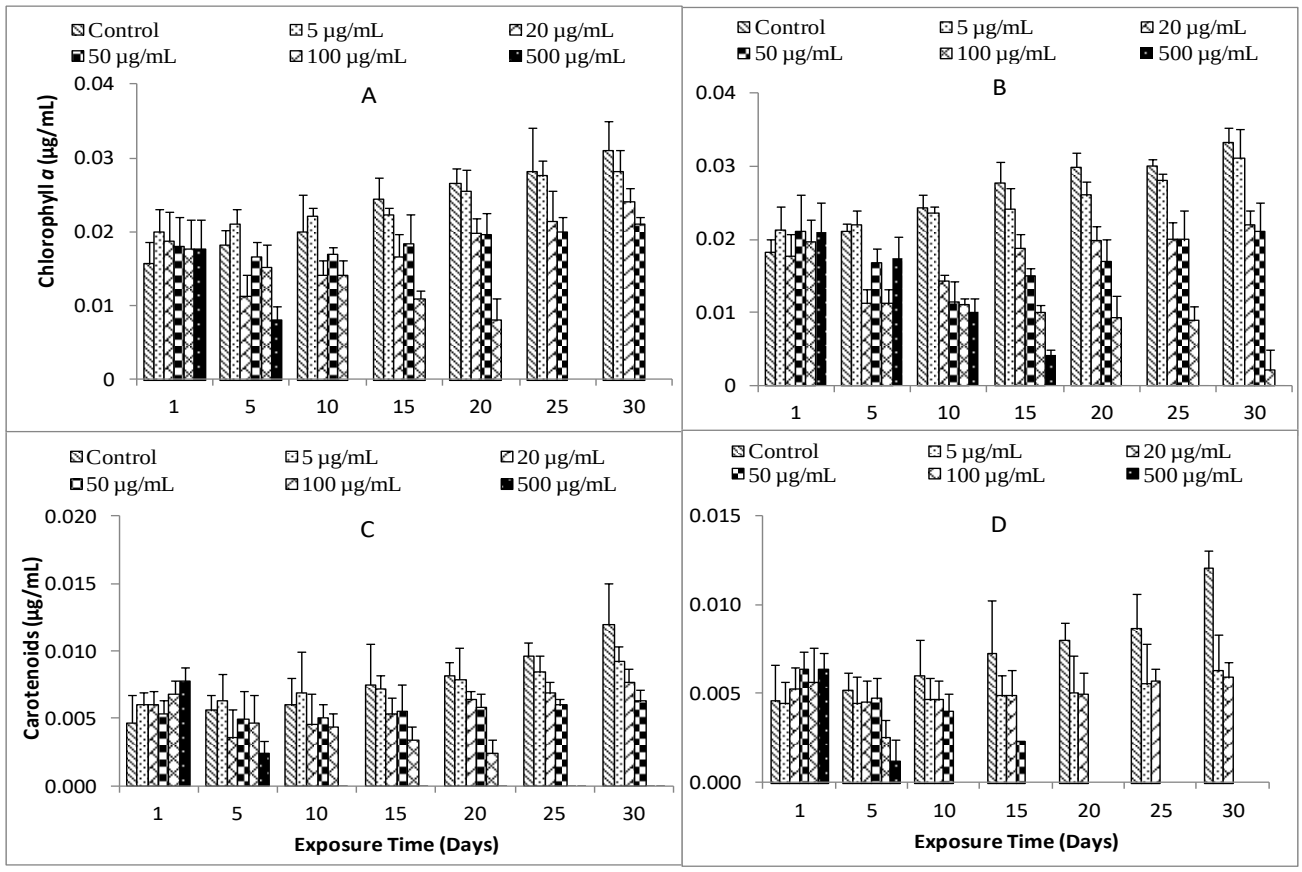

Fig. 2. Effect of Cypermethrin on (A) chlorophyll *a* of *Chroococcidiopsis* sp. (B) chlorophyll *a* of *Microcystis* sp. (C) Carotenoids of *Chroococcidiopsis* sp. (D) Carotenoids of *Microcystis* sp.

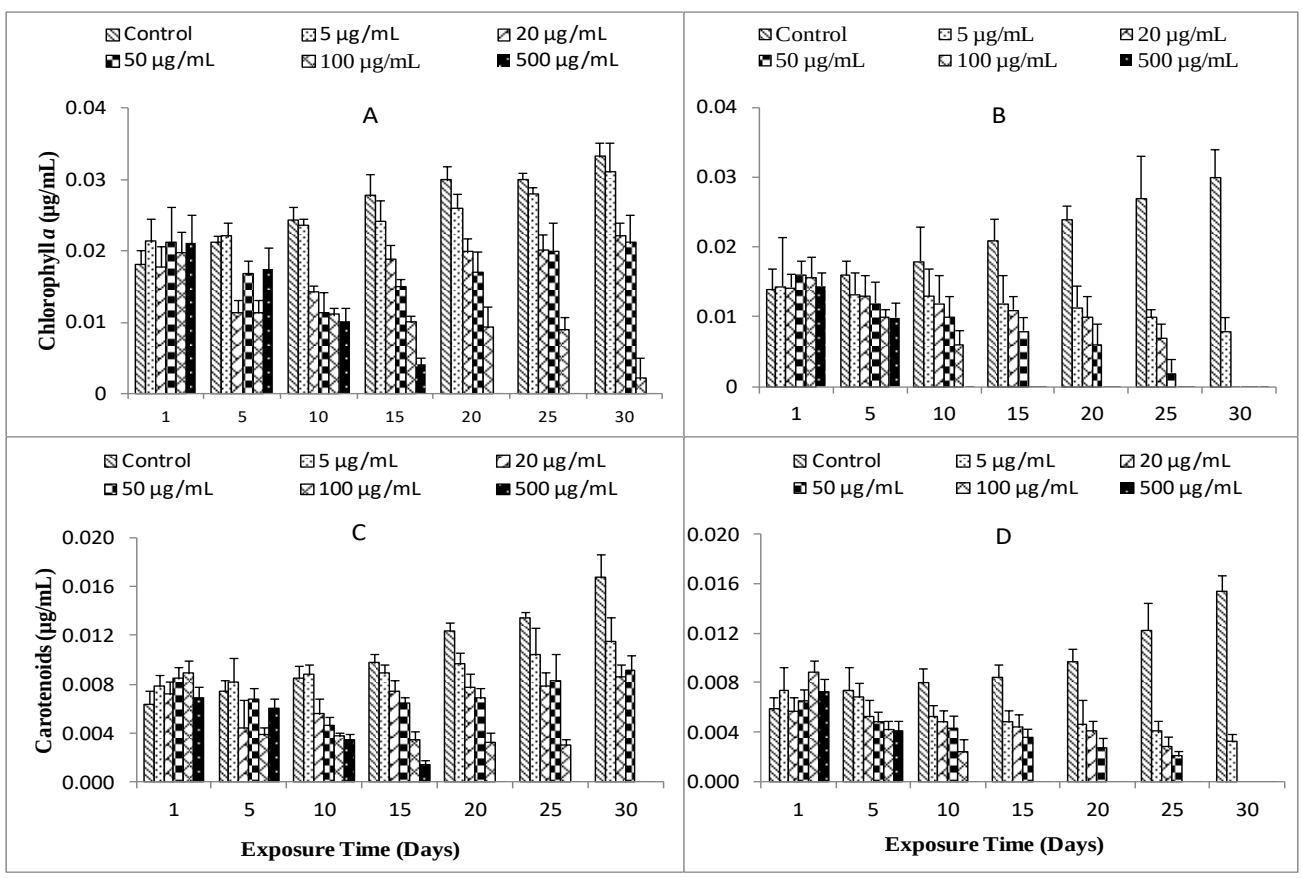

Fig. 3. Effect of Deltamethrin on (A) chlorophyll *a* of *Chroococcidiopsis* sp. (B) chlorophyll *a* of *Microcystis* sp. (C) Carotenoids of *Chroococcidiopsis* sp. (D) Carotenoids of *Microcystis* sp.

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