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ISOLATION AND SCREENING OF PESTICIDE RESISTANT CYANOBACTERIA FROM PESTICIDE CONTAMINATED FRESH WATER BODIES

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ABSTRACT

The accumulation of pesticide acethene was determined for freshwater photosynthetic microorganisms, the cyanobacterium *Oscillatoria curviceps*. An extremely rapid accumulation of pesticides acethene was recorded, although accumulation rate was lower for the lowest concentration of pesticides acethene when compared with higher concentrations of pesticide. Other parameters related to the pesticide concentration capacity of this cyanobacterium was also studied. Chlorophyll a, phycobilin pigments, carbohydrate, protein, lipid, amino acid, and pesticide accumulation analysis were performed. Growth was measured in terms of chlorophyll 'a' in all the six days in intervals. In control, growth was well pronounced up to 12 days from the day of inoculation, in other treatments (50,100,150,200 and 250 ppm) there was slight enhancement upto 12th days followed by a lag phase of another 18 days. Carbohydrate, protein, lipid and amino acid in cultures containing pesticides acethene were clearly affected by pesticide accumulation. Pesticide toxicity and microalgae sensitivity were used to determine the effectiveness of the bioaccumulation process and the stability of pesticide removal. *Oscillatoria curviceps* showed higher accumulation capability for this acethene pesticide. This study supports the usefulness of such cyanobacterium *Oscillatoria curviceps*, as a bioremediation technique in freshwater systems polluted with acethene pesticide compound.

Keywords: Acethene, Accumulation, Bioremediation, Cyanobacterium, *Oscillatoria curviceps*

INTRODUCTION

The use of pesticides has increased rapidly since World War II, and several recent studies show that pesticide residues frequently occur in surface water in agricultural areas (Ulen *et al* 2002). Pesticides used for agricultural activities have quite negative effects other than the target organisms they affect when turn back to the lakes or rivers by irrigation water or precipitation. When the studies on aquatic organisms analyzed, these studies are seen to be mostly concentrated on the organisms which represents the top level of food chain. By returning to the surface waters of the pesticides used for agricultural activities, one of the first organisms affected is the phytoplanktonic organisms constituting the first level in the food chain (Crosby, 1982).

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The existence and assess the level of the pollution, therefore simple and cheap methods are also needed to survey the environment for various pollutants, such as heavy metal, pesticides contaminations, heavy metals among others. Cyanobacteria contain effective homeostatic systems for maintaining the intracellular concentration of metal ions (Silver and Phung, 1996). If we compare many chemicals in terms of sensitivity, it is known that the sensitivity of algae and cyanobacteria are high (Aguilera-Del Real *et al* 1997). Therefore, in this study we have investigated the effect of various concentrations of pesticides acethene, changes in growth rate and photosynthetic capacities caused by various concentrations of pesticides acethene were measured in cultured cyanobacterium *Oscillatoria curviceps*.

Materials and methods

Test pesticides and cyanobacterium: The tested pesticides were purchased from local agroagencies in karur and obtained from the freshwater cyanobacterium *Oscillatoria curviceps* was used as test organisms in the present work. Cyanobacterium *Oscillatoria curviceps* was collected from fresh water in karur locality , purified, identified, and maintained under laboratory conditions.

The cultures were further maintained in the broth BG-11medium at low intensity of light 2000/ux and at 20^0C temperature. On the basis of morphological characteristics cyanobacterial cultures were identified at the genus level. The morphology of isolated cyanobacteria was observed under high power microscope. The identification of isolated cyanobacterium was done using the key given by (Desikachary, 1959), (Rippka *et al* 1979).

Growth measurements: To study the effect of pesticide on growth of cyanobacteria, the BG- 11 medium with different concentrations (Control, 50, 100, 150, 200 and 250 ppm) of pesticides were used. The flasks were inoculated and incubated at 24± 2ºC under 1800-2000 lux light intensity for 18 day. Effect of pesticide was observed on growth of cyanobacterium and measured in terms of its chlorophyll 'a'content (Mckinney, 1941).

The growth analysis viz., carotenoids, phycobilins, carbohydrates, proteins, total lipid and free amino acids content calculated at 24^{th} day after the inoculation. Estimation of phycobilins, estimation of carbohydrates followed by Dubois *et al* (1956), estimation of total proteins followed by Lowery *et al* (1951), estimation of total lipid followed by Sato and Murata, (1988), estimation of free amino acids followed by Jayaraman (1981).

Results and discussion

Growth was measured in terms of chlorophyll 'a' in all the six days in intervals. In control, growth was well pronounced up to 12 days from the day of inoculation, thereafter there was a gradual decline in growth till the end of 18^{th} days (Fig.1). In other treatments (50,100,150,200 and 250 ppm) there was slight enhancement upto 12 days followed by a lag phase of another 6 days. Thereafter gradual increase upto the 12^{th} days and showed a gradually decrease upto 18^{th} day. Maximum growth was observed in control and minimum was noticed at 250 ppm on the 12^{th} day. The present results are also in consonance with the deleterious effects of other fungicides on chl-a and carotenoids of marine microalgal communities investigated by Porsbring *et al* (2009). Anilofos inhibited all photosynthetic pigments of *Anabaena torulosa* (Singh *et al* 2013), *Oscillatoria simplicissima* Singh and Sandhu, (2010).

Among the phycophilins and C-phycocyanin pigments were recorded in all the concentrations including control. C-phycocyanin was not observed in the test organism. There was a greater reduction in the level of phycophilins noticed at the highest concentration (250ppm) of pesticide on the 18^{th} day and the lesser reduction at 50 ppm as compared to control (Fig.2). In general, as the concentration of pesticide increases, the phycophilins level decreased considerably. The findings are also in agreement with Mostafa and Helling (2002) who suggested that drop in chlorophyll-a, and phycobiliprotein contents might be ascribed to the inhibition of pigment synthesis directly by the insecticide or accelerated degradation of pigments due to increased Active

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Oxygen Species (AOS) formation at various sites of the photosynthetic electron transport chain during stress. Treatment of *Anabaena cylindrica* with Bentazon (2 mM) for 72 h decreased total phycobiliproteins by 58% where as Molinate at highest tested concentration (2 mM) completely suppressed phycobiliproteins after 24 h treatment (Galhano *et al* 2010).

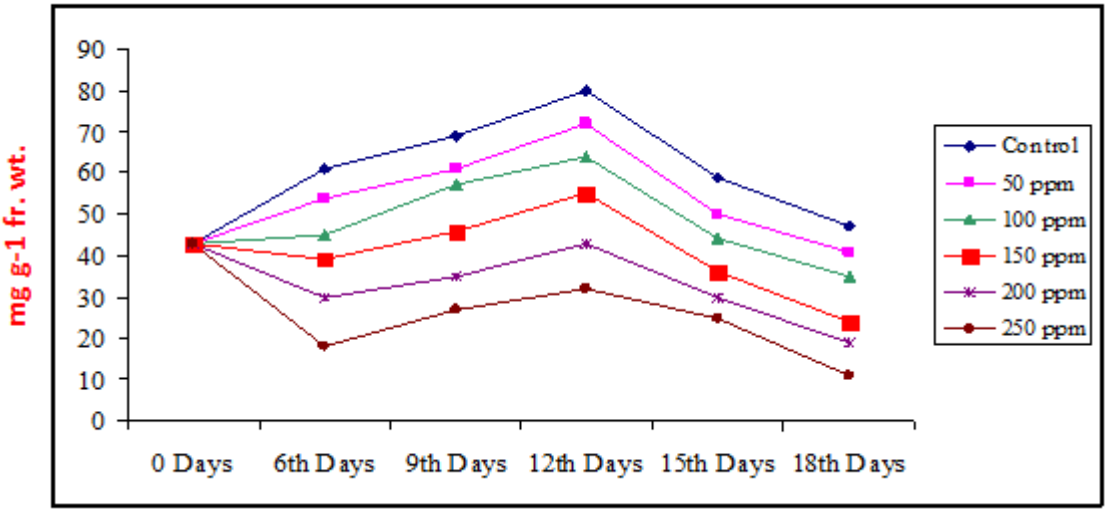

Figure 1: Effect of different concentration of pesticide on chlorophyll 'a' of *O. curviceps*

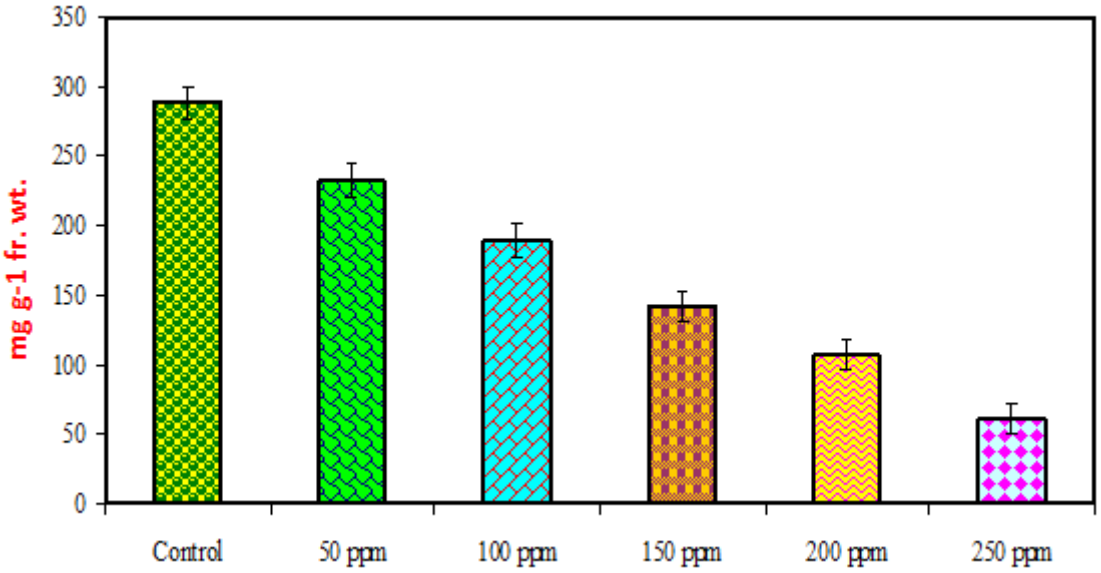

Figure 1: Effect of different concentration of pesticide on carbohydrate of *O. curviceps*

Comparison carbohydrate of *Oscillatoria curviceps* estimated at the different concentration of pesticide acethene was shown in figure 3 to 6. There was a greater reduction in the level of carbohydrate noticed at the highest concentration (250ppm) of pesticide on the 18th day and the lesser reduction at 50ppm as compared to control (Fig.3). In general, as the concentration of pesticide increases, the carbohydrate level decreased considerably. These results have been well corroborated with those of Kumar *et al* (2008). The cyanobacterium *Oscillatoria pseudogeminata* was conducted in the laboratory to test the effect of carbaryl on

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biochemical components such as carbohydrate and protein contents were also decreased gradually with increasing concentration of pesticides reported by Bakiyaraj *et al* (2014).

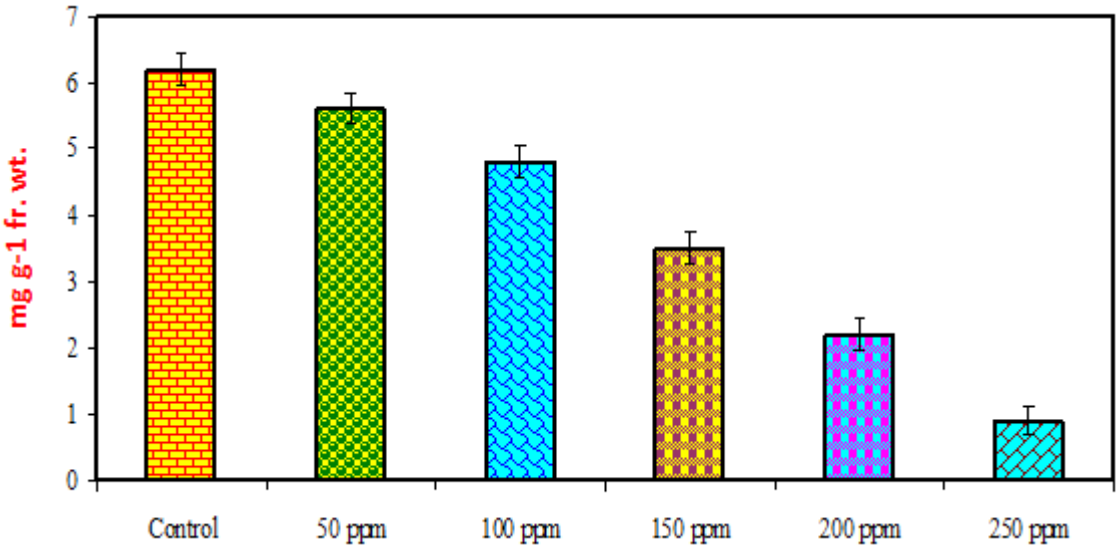

Figure 3: Effect of different concentration of pesticide on phycophilins of *O. curviceps*

As can be seen from the protein content was maximum in control, Apart from control, all the concentration showed gradual reduction in protein content. Nearly reduction of protein was observed in *Oscillatoria curviceps* treated with 250 ppm, but in the lowest concentration (50 ppm) minimum reduction was noticed as compared to control (Fig. 4). The results were further confirmed by Shehata *et al* (2001). Supplementation of Butachlor (8-20 mg L-1) in culture medium of *Nostoc muscorum* resulted in decrease of protein content by 27-89% whereas 14 and 63% decrease in protein content in 5 and 8 mg Thiobencarb L-1 was reported (Dowidar *et al* 2010). Carbaryl (40 mg L-1) decreased content of protein by 40% in a paddy field cyanobacterium *Calothrix brevissima* (Habib *et al* 2011).

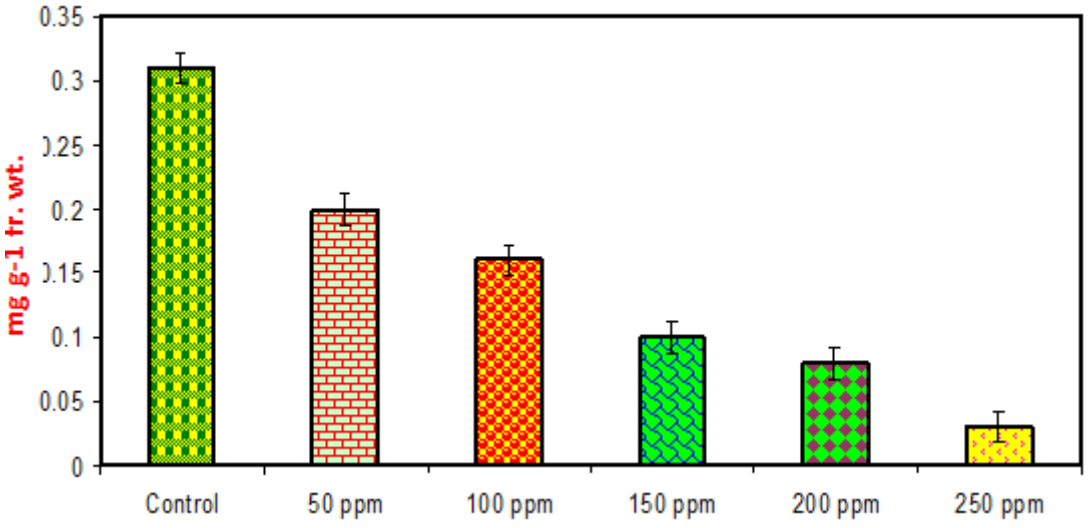

Figure 4: Effect of different concentration of pesticide on protein content of *O. curviceps*

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Lipid level was decreased drastically in treated increasing pesticide concentration except control. The highest reduction and the lowest reduction of lipids were observed at 250 ppm and 50 ppm respectively (Fig.5). The maximum reduction was found at 250 ppm and minimum reduction was found at 50 ppm were also decreased gradually with increasing concentration of pesticide. Likewise *Westiellopsis prolific* treated with 2,4-D showed maximum reduction of carbohydrate, protein and lipid content in all concentration (Muruganantham and Manoharan, 1998)

Amino acid was decreased drastically in treated increasing pesticide concentration except control. The highest reduction and the lowest reduction of amino acid were observed at 250 ppm and 50 ppm respectively, (Fig.6) also decreased gradually with increasing concentration of pesticide. Muruganantham (2001) also observed the decrease in amino acid content with increasing concentration of heavy metal copper. *Oscillatoria pseudogeminata* was conducted in the effect of carbaryl on biochemical components such as carbohydrate and protein contents were also decreased drastically with increasing concentration of pesticides reported by Bakiyaraj *et al* (2014).

Accumulation percentage of the pesticide was found to be the highest at 50 ppm (69%) soil level Fig 7. Accumulation of cyanobacterium (*Oscillatoria curviceps*) (21%) was the lowest at 50 ppm pesticide level in the soil. Similar algal scrubber is also used as water filtering device in aquariums to remove undesirable chemicals and organic compounds from the water reported by (Mulbry *et al* 2008; Adey *et al* 2011).

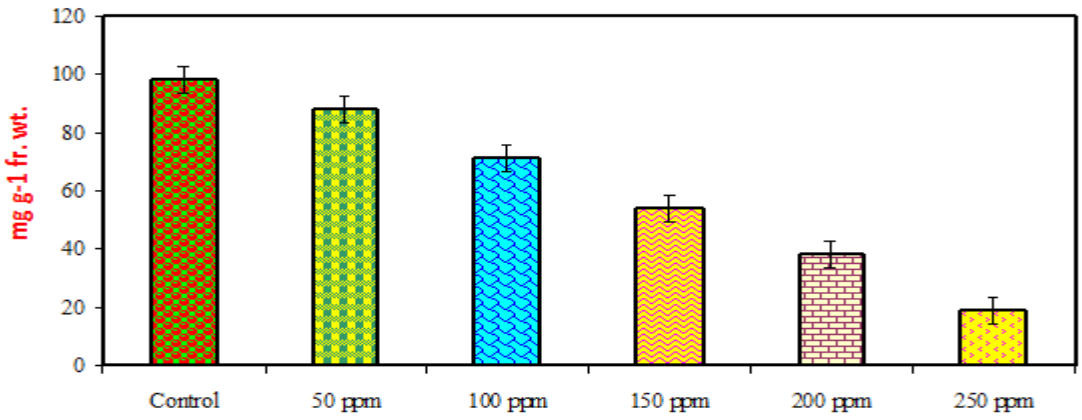

Figure 5. Effect of different concentration of pesticide on Lipid content of *O. curviceps*

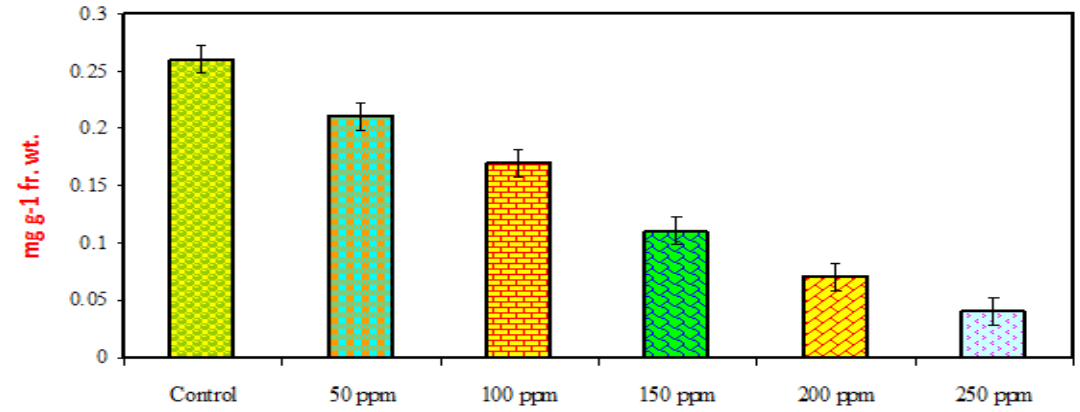

Figure 6: Effect of different concentration of pesticide on amino acid of *O. curviceps*

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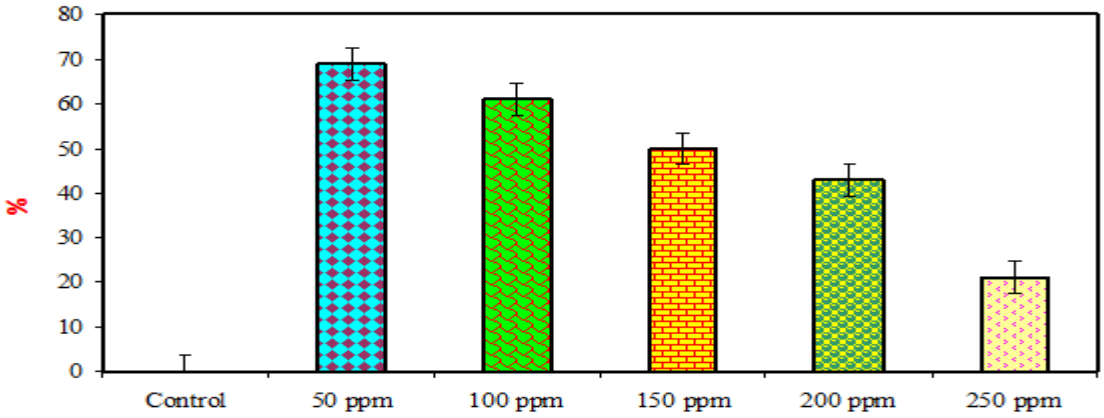

Figure 7: Accumulation of pesticide by cyanobacteria (*Oscillatoria curviceps*)

CONCLUSIONS

The experiments was conducted with a view to determining the deleterious and differential effects of pesticides on the photosynthetic pigments, biochemical and accumulation of cyanobacterium. The results suggest that *Oscillatoria curviceps* was the most susceptible organism to the pesticide degradation. Based on these observations dominance of non-heterocystous genera over heterocystous cyanobacterial genera was confirmed with earlier reports which might play a unique role in bioremediation of pesticide toxicity.

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