Toxic Effect of Chlorpyrifos and Dimethoate on Protein and Chlorophyll-a Content of *Spirulina Platensis*

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Abstract: **To increase crop productivity pesticides are widely used in agriculture and indirectly affecting flora and fauna. From agricultural fields pesticides reach to different water resources and cause pollution. *Spirulina platensis* having high protein content and good supplement for human food and animal feed was found in aquatic environment. Thus the cells of *Spirulina* were treated with different concentrations (0.5, 1, 5, 10 and 15 ppm) of chlorpyrifos and dimethoate to find effect on pigment and protein content. The most stimulatory effect on pigment and protein content was observed at 15.0 ppm, for this concentration chlorophyll-a and protein was almost inhibited. Low concentrations such as 0.5 ppm of pesticides was not observed toxic towards chlorophyll-a and protein. As the concentration increased above 1.0 ppm, pigment and protein content start decreasing. Thus concentration above 1.0 ppm showed toxicity towards *Spirulina platensis*. In this way the pesticides used in agriculture should be used at definite concentration to protect this non target organism.**

Keywords— Spirulina platensis, pesticides, chlorophyll-a, protein, environment

I. INTRODUCTION

Contamination of water by increased usage of pesticides becomes a serious environmental problem. When the pesticides are used in agricultural fields, they go through adsorption, volatilization, chemical and microbiological transformation causing adverse effect on aquatic ecosystem. Pesticides residues are found in surface and ground water causing harmful effect on non- target organisms **[1],[7]**. Although organophosphorus (Chlorpyrifos and dimethoate) pesticides are biodegradable in nature but their residues inhibit the activity of acetylcholinesterase that is a neurotransmitter. **[4].**

Spirulina platensis is a cynobacterium photosynthetic, multicellular blue green algae found in aquatic environment. It is an excellent source of proteins (60-70%), vitamins, fatty acids, minerals and chlorophyll-a. Due to its nutritional, ecological and therapeutic importance *Spirulina* has been the focus of intensive research **[**2],[3],[8].

In the present work *Spirulina platensis* have been cultured in the laboratory for different pesticides concentrations. The fast growing *Spirulina platensis* cultures were selected to assess the effect of chlorpyrifos and dimethoate pesticides on protein and chlorophyll-a content.

II. MATERIALS AND METHODS

A. Organism

Spirulina platensis was obtained from department of Biotechnology, Jiwaji University, Gwalior (M.P.) India. The culture was maintained in Zarrouk's medium **[9]** in culture room at 30˚C and illuminated fluorescent tubes light (at surface of the vessels with 12/12 dark–light rhythm).

B. Incubation and maintenance of culture

Spirulina was grown in the Zarrouk's medium (ZM) and $NaHCO_3$ was added after autoclaving and pH was adjusted to 9 with 1N NaOH. Different pesticides concentration (0.5, 1, 5, 10 and 15 ppm) was added directly to the basal medium. Exponentially growing culture was inoculated and maintained in culture room. All the treated *spirulina* cultures were under observation till 6^{th} day. All the experiments were done in triplicate including control and flasks were shaken twice a day manually.

The chlorophyll-a was estimated **[6]** and the absorbance of the cell free metabolic extract was recorded at 663 nm against methanol as a blank. protein analysis were done with Lowry method **[5]** The absorbance of the clean liquid was measured at 650nm, and the amount of algal cell. Protein calculated as mg/l culture with reference to a standard-calibrated curve, obtained with BSA.

The significance of differences between mean values was determined by paired t-test at Confidence level of 95 % and p value 0.05 was preferred.

III. RESULTS AND DISCUSSION

A. Effect of chlorpyrifos on chlorophyll-a content

The exposure to chlorpyrifos reduced the chlorophyll-a content of *Spirulina platensis* (Fig.1). At concentration 0.5

ppm chlorpyrifos reduced the chlorophyll content from 12.02 mg/l in the untreated sample to 10.88 mg/l. The reduction increased at 1.0 ppm and 5.0 ppm chlorpyrifos to 10.27 mg/l and 6.08 mg/l, respectively. Concentration 10.0 ppm and 15.0 ppm reduced the chlorophyll content to 2.96 mg/l and 2.71mg/l, respectively.

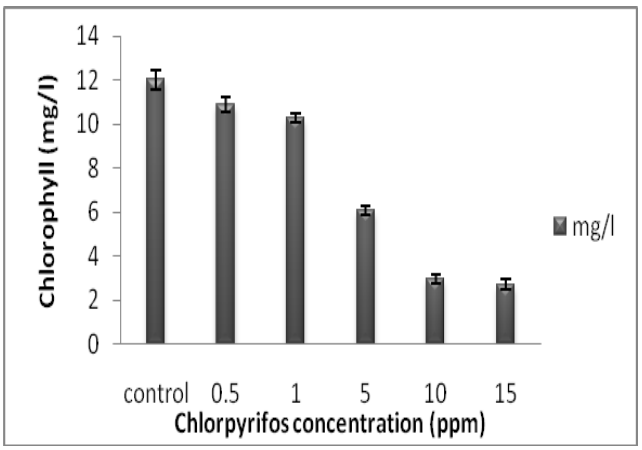

Fig.**1**. Effect of chlorpyrifos on chlorophyll-a content of *S.platensis*.
(All data represent the mean of three replicates. Bars represent ±SD)

B. Effect of dimethoate on chlorophyll-a content

The chlorophyll content also was affected by different concentrations of dimethoate (Fig.2). The first dimethoate concentration 0.5 ppm reduced the chlorophyll content from 15.34 mg/l in control to 13.67 mg/l and 1.0 ppm reduced it to 10.21 mg/l, but 5.0,10.0 ppm and 15.0 ppm concentrations reduced the chlorophyll to 5.79 mg/l, 3.26 mg/l and 1.76 mg/l, respectively.

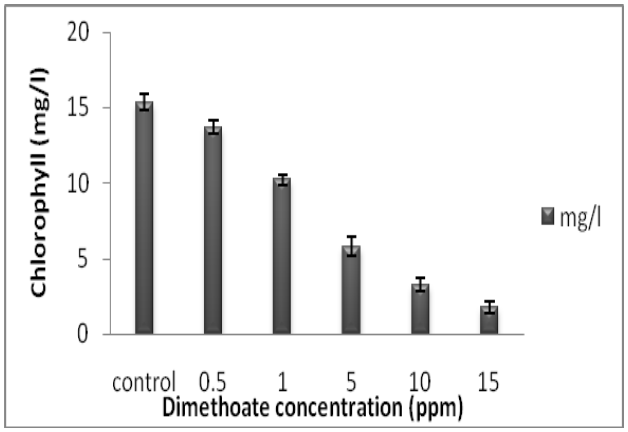

Fig.**2** Effect of dimethoate on chlorophyll-a content of *S.platensis*
(All data represent the mean of three replicates. Bars represent ±SD)

The major component of the photosynthetic light-harvesting system of *S.platensis* is chlorophyll *a* in the thylakoid membranes. The results indicated that chlorophyll content decreased by increasing the concentration of pesticides. The concentration above 1.0 ppm significantly ($p < 0.05$) reduced the chlorophyll-a content when compared with control. Thus chlropyrifos and dimethoate showed toxicity towards *Spirulina platensis*.

C. Effect of chlorpyrifos on total protein content

The total protein content of *S.platensis* was affected upon exposure to 0.5, 1.0, 5, 10 and 15.0 ppm of chlorpyrifos (Fig.3). Compared to the total protein content of the untreated sample (316.55 mg/l), the total protein contents at concentrations 0.5, 1.0,5.0,10.0 and 15.0 ppm were (348.27 mg/l), (317.24 mg/l), (197.17 mg/l),(72.06 mg/l) and (73.10 mg/l) respectively. Hence the present observation concludes that at 0.5 ppm and 1.0 ppm there is no effect on protein content, while 5.0, 10.0 and 15.0 ppm significantly ($p < 0.05$) reduced the protein content. In this way chlorpyrifos were found toxic for *Spirulina.*

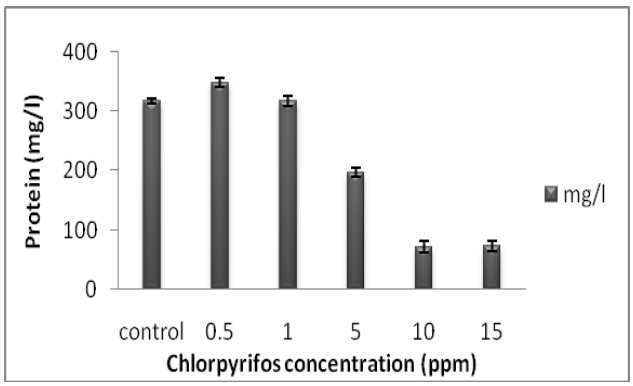

Fig.**3** Effect of chlorpyrifos on total protein content of *S.platensis*.
(All data represent the mean of three replicates. Bars represent ±SD)

D. Effect of dimethoate on total protein content

The total protein content of *S.platensis* was affected upon exposure to 0.5, 1.0, 5, 10 and 15.0 ppm of dimethoate (Fig.4). Compared to the total protein content of the untreated sample (597.24 mg/l), the total protein contents at concentrations 0.5, 1.0,5.0,10.0 and 15.0 ppm were (563.44 mg/l), (486.89 mg/l), (286.20 mg/l),(214.13 mg/l) and (150.34 mg/l) respectively. Hence the present observation concludes that 5.0, 10.0 and 15.0 ppm were found toxic and significantly ($p < 0.05$) varying with control.

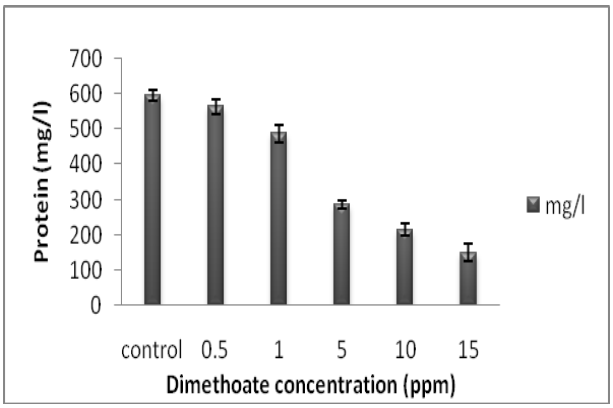

Fig.4. Effect of dimethoate on total protein content of *S.platensis*.
(All data represent the mean of three replicates. Bars represent ±SD)

The relationship between the two patterns was quite logic, because any changes in the chlorophyll content or production will influence the photosynthesis process , which in turn will affect the primary products needed for protein synthesis.

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