International Journal of Geology, Agriculture and Environmental Sciences
Volume – 3 Issue – 6 December 2015
Website: www.woarjournals.org/IJGAES *ISSN: 2348-0254*

Toxicity of Organophosphorus pesticides on ricefield Cyanobacteria

D. Sahu[1], A. K. Bastia[2], B. Rath[1]

[1] Department of Biotechnology, North Orissa University,
Sriram Chandra Vihar,Takatpur, Baripada-757003,Odisha.

[2]Department of Botany, North Orissa University,
Sriram Chandra Vihar, Takatpur, Baripada-757003, Odisha.

Abstract: ***The present study revealed the organophosphorus insecticide (Monocrotophos and Chloropyriphos) induced changes in biochemical parameters related to photosynthetic pigments and protein content of two cyanobacteria (Nostoc commune and Anabaena variabilis) isolated from local rice fields of Mayurbhanj district in Odisha and grown under laboratory conditions at Department of Biotechnology, North Orissa University,Baripada,Odisha. Differential response on growth, photosynthetic pigments (chlorophyll a and carotenoids) and protein content was observed on both the test species to selected concentration (EC25, EC50 and sublethal at which 25 and 50 percent growth was reduced and at sublethal dose the growth of the organism was least as compared to control) of two organophosphorus insecticide treatment at different days of incubation (4,8,12 and 16 days).The experimental result infers that the toxic effect of both the insecticides was found only after 8days of incubation in both the species. The deleterious effect of chloropyriphos was significant as compared to monocrotophos in both the species at different days of incubation. Among both the species Nostoc commune was found to be more resistant to both the insecticides. The toxic effect of both the insecticides at EC25 and EC50 dose was more pronounced on 16 days old culture showing decrease in growth, pigment and protein content on both the test species. However the toxic effect of sublethal dose of both the insecticides inhibited growth on 12 and 16 days old culture. Among both the insecticides Monocrotophos was found to have more deleterious effect on both the species than that of Chloropyriphos and this trend was observed to be more pronounced on long period of incubation as compared to short period of incubation.The results so obtained concludes that the toxic effect of insecticides became harmful to the nontargeted cynobacteria in rice fields if retained for a longer time. On the other hand the deleterious effect can be minimized if the rice fields are flooded or irrigated after application of insecticides so that the pest were killed on immediate exposure without any adverse effect on cyanobacteria of the same habitat.***

Keywords: Cyanobacteria, incubation, pesticide, paddy fields, toxicity.

1. Introduction

Cyanobacteria, a group of ubiquitous, photosynthetic prokaryotes which perform two key biological processes such as oxygenic photosynthesis and nitrogen fixation together in same the cells/filaments, and enrich the paddy soil particularly with nitrogen and humus contents [1], [2]. Cyanobacteria are exposed to various types of natural stresses, such as nutrient limitation, pesticides, pollution, drought, salinity, temperature, pH, light intensity and quality, etc. Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no or low cost [3] . Extensive and regular use of pesticides in modern rice cultivation is reported to adversely affect the diversity, biology or even sustainability of cyanobacteria often leading to their complete elimination from the field [4], [5].

The economy of developing countries like India is agricultural based, but pests act as main challenge in maintaining the economy. So, different pesticides are being used by the farmers for so many years all over the world. The various classes of pesticides include the organophosphates, carbamates, pyrethroids, organochlorines etc [6], [7].

The Organophosphorus insecticides are most widely applied in crops due to its broad spectrum of activity and low cost. The application of insecticides, a group of pesticides, in crop fields for selective control of pests in the modern age has led to serious environmental contamination resulting in greater loss of crop productivity and growth of many beneficial micro-organisms [8]. The cyanobacteria are exposed to insecticides which are indispensable to the modern agricultural practice. However, the use of these insecticides over the years has resulted in problems caused by their interactions with the biological systems in the environment and has deleterious effects on cyanobacteria [9] .

Though a considerable amount of work relating to the insecticide induced inhibitory effects on growth, photosynthetic pigment contents and nitrogen fixation in cyanobacteria has been done but little work has been done on insecticides particularly organophosphorus induced effects on growth, pigment and protein content. However, agrochemical residue present in soil is likely to inhibit the biofertilizer potential of cyanobacteria depending upon the dose and time of exposure and individual characteristics of the organism**.** Perhaps, it is evident that many organophosphorus insecticides at the recommended field application have none or accelerating effect on growth of cyanobacteria but may affect various physiological processes in cyanobacteria [10], [11] . As every insecticide used in agricultural practices affects the growth of non target soil microorganisms depending on the period of exposure, therefore the aim of this work was to establish the differential toxicity effects of the two selected rice field insecticides (Monocrotophos and Chloropyriphos) on growth and survivability potentials on *Anabaena variabilis* and *Nosctoc commune* isolated from local rice fields of Mayurbhanj district in the state of Odisha.

2. Materials and Methods

1.1. Glasswares

Borosil make glass vessel were used throughout the experimental work. Hard glass test tubes (15x150mm) and 100ml conical flask were used for culturing the test organisms. The test tubes and conical flasks were stopper by non absorbent cotton.

1.2. Test culture media

The BG11 media was prepared from the stock solution present in laboratory. p^H of the culture medium before autoclaving was always maintained at 7.5 to 7.6**.** Analytical grade chemicals of SRL, MERCK and HIMEDIA and glass distilled water was used for the preparation of culture media.

1.3. Test Organisms

Experimental strains, *Nostoc commune* and *Anabaena variabilis* are two diazotropic filamentous cyanobacteria, collected from some local paddy field soils of Mayurbhanj district of Odisha state. These were isolated and cultured in BG 11 media under controlled laboratory condition and maintained in Department of Biotechnology, North Orissa University, Baripada, Mayurbhanj, Odisha.

1.4. Test Insecticides

Two organophosphorus insecticides Monocrotophos and Chloropyriphos of analytical grade was used at selected dose (EC25,EC50 and Sublethal at which 25%, 50% and least growth was observed respectively as compared to control).The EC25,EC50 and sublethal dose of Monocrotophos and chloropyriphos for *Nostoc commune* was 0.05, 0.5,2 µl/ml and 0.05,0.5 and 1 µl/ml and for *Anabaena variabilis* it was 0.1,0.3,1 µl/ml and 0.05,0.3 and 0.7 µl/ml respectively. Samples were taken after every four days up to 16 days for assay of growth, chlorophyll-a, carotenoid and protein content.

1.5. Culture media and culture conditions

Experiments were conducted in 15 × 150mm hard glass test tubes containing 10ml of nitrogen free BG_{11} medium with or without various concentration of insecticide Monocrotophos and Chloropyriphos and 1ml of homogenized suspension of the organisms. Experimental cultures were incubated at 26 ± 1^0C under 3000 lux light intensity. The cyanobacterial cultures were maintained in a culture room at a temperature of 25 ± 1° C and 3000 lux light intensity with a photoperiod of 16h light and 8h dark at four days interval of time up to 16 days. Each treatment was of three replicates. The liquid cultures in the flask and tubes were hand shaken daily 2- 3 times to provide uniform light, aeration and nutrient to the suspension culture and to avoid sticking of cyanobacterial cell to the walls of the glass vessel which may result in uneven growth and subsequent experimental error.

1.6. Test methods

1.7.

1.7.1.Growth Measurement

After every 4 days of incubation 5ml of cultured algal samples were taken for measurement of growth by spectrophotometer at 760nm, [12].

1.7.2.Chlorophyll Estimation

The chlorophyll content was estimated following the method of Mackinney [13] by taking the absorbance at 663nm in a systronics make spectrophotometer (model.110)

1.7.3.Carotenoid Estimation

The carotenoid content was measured by taking the absorbance at 470nm and was estimated following the method of Davis [14].

1.7.4.Protein estimation

The Protein content was estimated following the method of Lowry et,al.[14] by taking the absorbance of the sample at 700nm.

1.8. Statistical evaluation

The experiments were set up in triplicates for each treatment and mean of such data was presented with the standard deviation.

3. Results

Effect of EC25, EC50 and sublethal dose of Monocrotophos corresponding to 0.05,0.5,2 µl/ml for *Nostoc commune* and 0.1,0.3,1 µl/ml for *Anabaena variabilis* and similarly Chloropyriphos at 0.05,0.5,1µl/ml for *Nostoc commune* and 0.05,0.3,0.7 µl/ml for *Anabaena variabilis* was used to analyse growth characteristics, pigment and protein content at different days (4^{th}, 8^{th}, 12^{th} and 16^{th} day) to know the toxicity of the agrochemical on the non target organism. Study on Growth response of *Nostoc commune* when treated with Monocrotophos has shown increasing trend on all the days of incubation at control , EC25 , EC50 dose of the insecticide whereas at sublethal dose growth was increased upto 4 days followed by significant decrease at 16 days of incubation (fig. la).

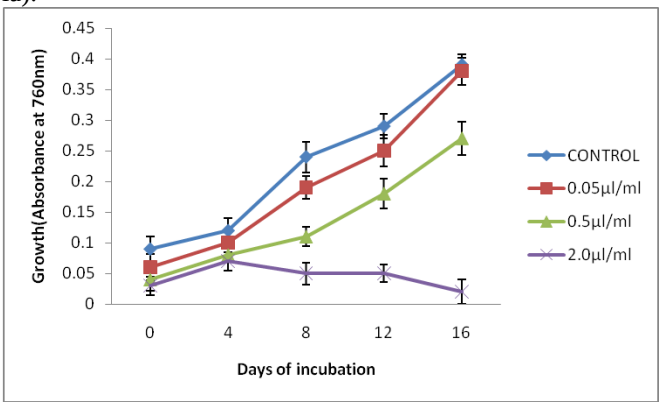

Figure 1a: Effect of various concentration of Monocrotophos on the Growth of *Nostoc commune*.

However in *Anabaena variabilis* growth was increased upto 12 days, 8 days and 4 days at EC25, EC50 and sublethal dose of Monocrotophos respectively followed by sudden decrease on 16 days old culture (fig. 3a). On the other hand effect of Chloropyriphos on *Nostoc commune* was found to be inhibitory after 4 days of incubation in all the test concentration (fig. 2a),but growth response of *Anabaena variabilis* was found to be stimulated in all the test concentration of the insecticide upto 16 days of incubation (fig 4a).

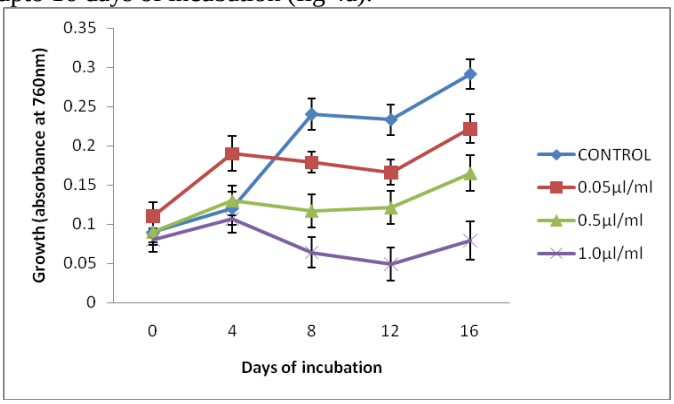

Figure 2a: Effect of various concentration of Chloropyriphos on the Growth of *Nostoc commnue*

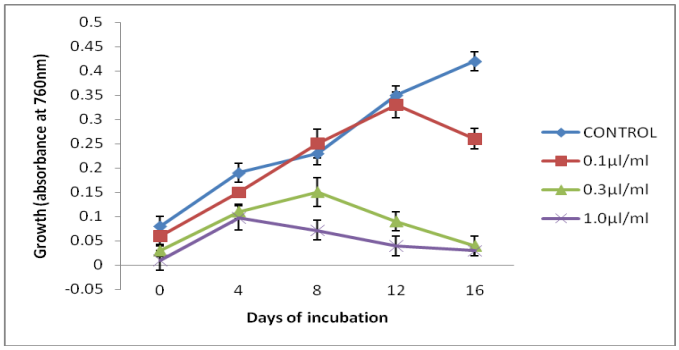

Figure 3a: Effect of various concentration of Monocrotophos on the Growth of *Anabaena variabilis*

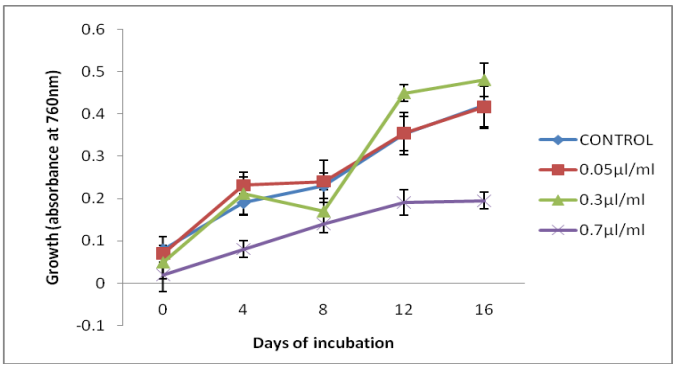

Figure 4a: Effect of various concentration of Chloropyriphos on the growth of *Anabaena variabilis*

Cellular chlorophyll-a content varied when *Nostoc commune* was treated with two insecticides (Monocrotophos and Chloropyriphos). The chlorophyll-a content was found to increase upto 12 days of incubation followed by immediate decrease on 16 days old treated culture in EC25, EC50 dose of both the insecticides in *Nostoc commune* (fig. 1b, 2b).

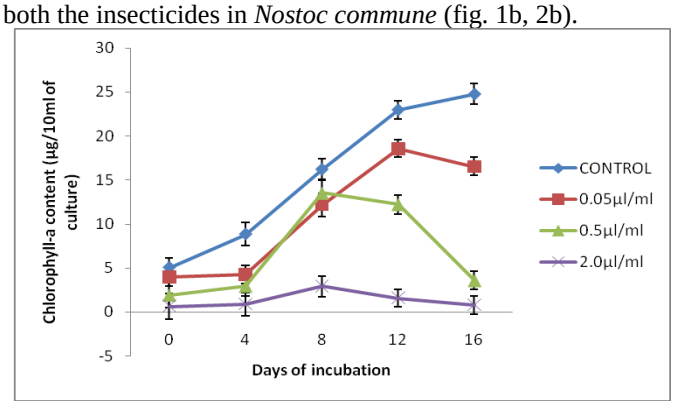

Figure 1b: Effect of various concentration of Monocrotophos on the chlorophyll-a content of *Nostoc commune*.

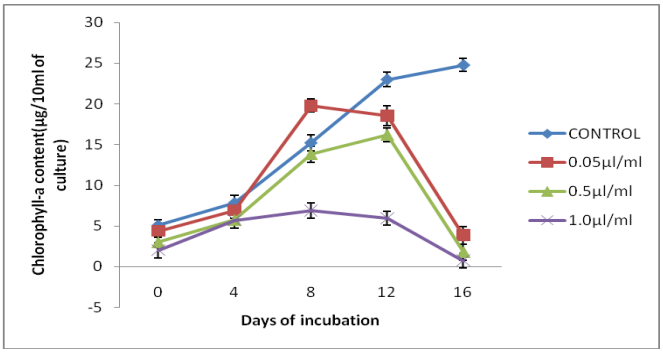

Figure 2b: Effect of various concentration of Chloropyriphos on the Chlorophyll-a content of *Nostoc commnue*.

The chlorophyll-a content in *Anabaena variabilis* when treated with Monocrotophos in the culture media showed similar result like that of *Nostoc commune* (fig. 3b).

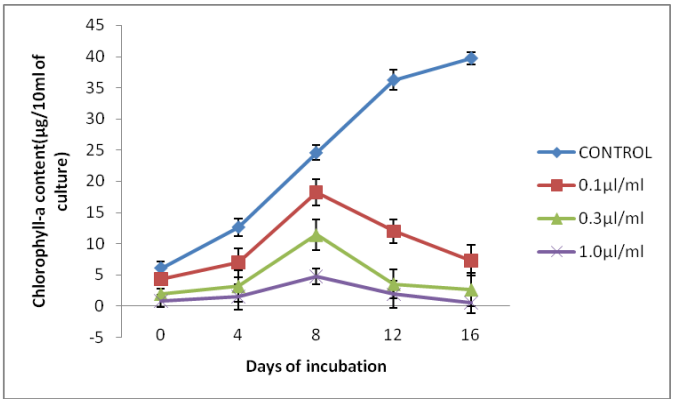

Figure 3b: Effect of various concentration of Monocrotophos on the Chlorophyll-a content of *Anabaena variabilis.*

However Chloropyriphos enhanced the chlorophyll-a content in *Anabaena variabilies* at EC25 dose in all the days of incubation and sublethal dose found to be toxic by reducing the chlorophyll-a content in all the days of incubation period (fig. 4b).

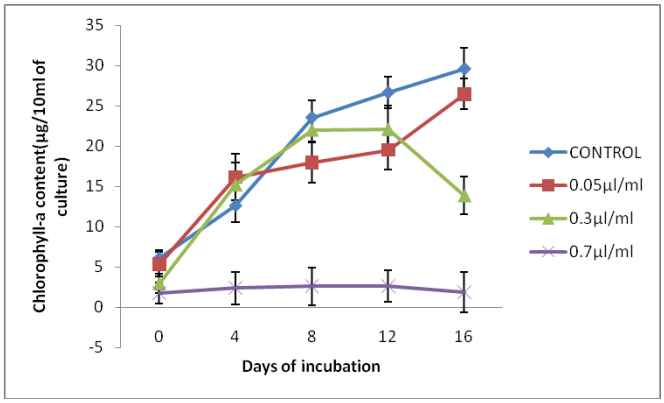

Figure 4b: Effect of various concentration of Chloropyriphos on the Chlorophyll-a content of *Anabaena variabilis.*

The carotenoid content was increased upto 8 days at control, EC25, EC50 and sublethal dose of both the insecticides followed by decrease in *Nostoc commune* (fig.1c, 2c).

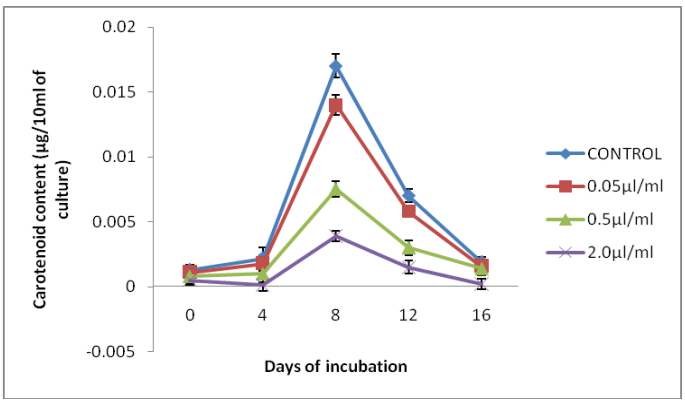

Figure 1c: Effect of various concentration of Monocrotophos on the Carotenoid content of *Nostoc commune*

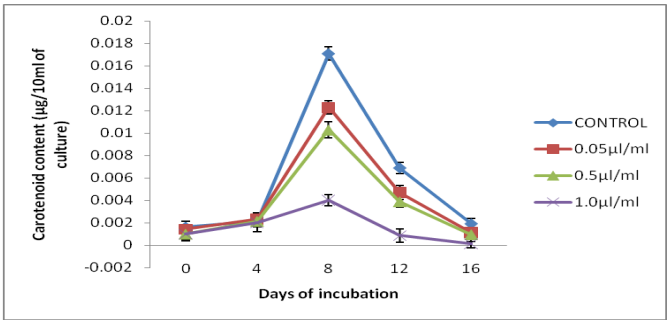

Figure 2c: Effect of various concentration of Chloropyriphos on the Carotenoid content of *Nostoc commnue*

However in *Anabaena variabilis* treated with different dose of Monocrotophos showed increase in carotenoid content upto 8 days followed by decrease upto 12 days and then little increase on 16 days old culture (fig.3c).

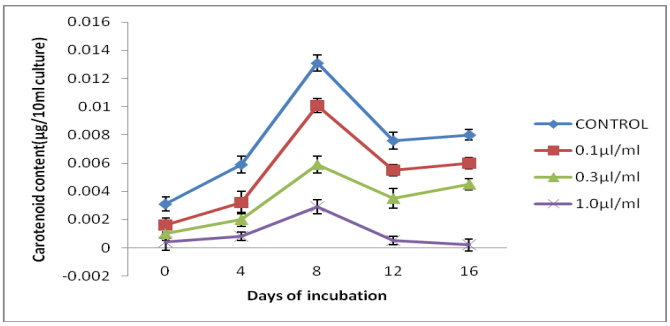

Figure 3c: Effect of various concentration of Monocrotophos on the carotenoid content of *Anabaena variabilis.*

The toxcic effect of Chloropyriphos on *Anabaena variabilis* showed differential response on all the test concentration. The carotenoid content was reduced only in EC50 and sublethal dose on prolonged period of incubation (fig. 4c).

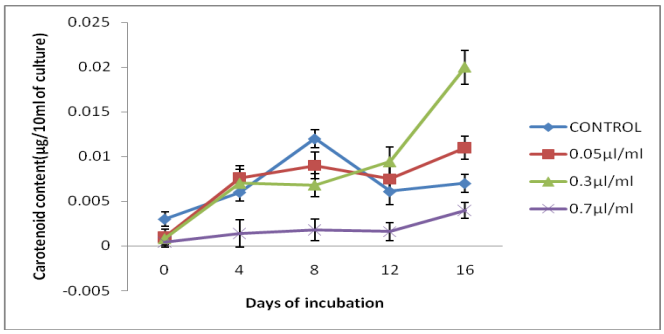

Figure 4c: Effect of various concentration of Chloropyriphos on the Carotenoid content of *Anabaena variabilis*

The protein content in *Nostoc commune* was inhibited at EC25 dose of Monocrotophos on 16 days old culture whereas at other test concentration the protein content was increased from 4 to 16 days (fig.1d).

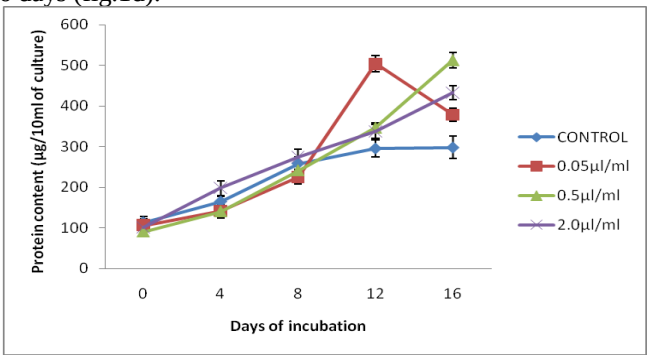

Figure 1d: Effect of various concentration of Monocrotophos on the protein content of *Nostoc commune*

However protein content was adversely affected when both the test species were treated with Choloropyriphos at EC25, EC50 and sublethal dose after 8 days of incubation period (fig. 2d).

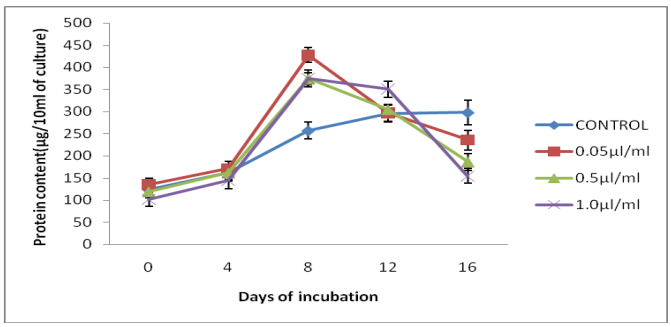

Figure 2d: Effect of various concentration of Chloropyriphos on the Protein content of *Nostoc commnue*

In *Anabaena variabilis* protein content was reduced at EC25 dose of Monocrotophos after 8days of incubation whereas protein content was increased upto 12 days followed by decrease in EC50 and sublethal dose (fig. 3d).

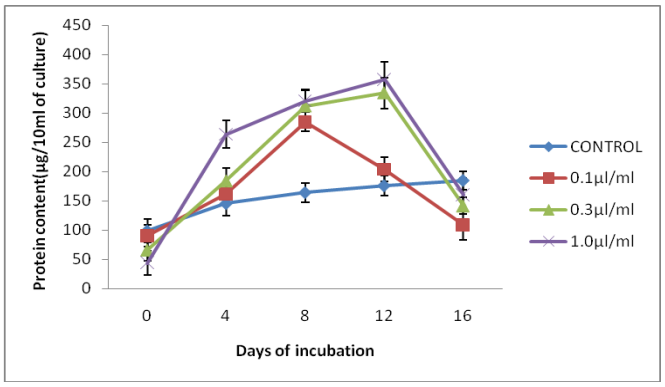

Figure 3d: Effect of various concentration of Monocrotophos on the Protein content of *Anabaena variabilis.*

On contrary to this in *Anabaena Variabilis* protein content was increased upto 8days followed by decrease at EC25, EC50 and Sublethal dose of Chloropyriphos (fig. 4d).

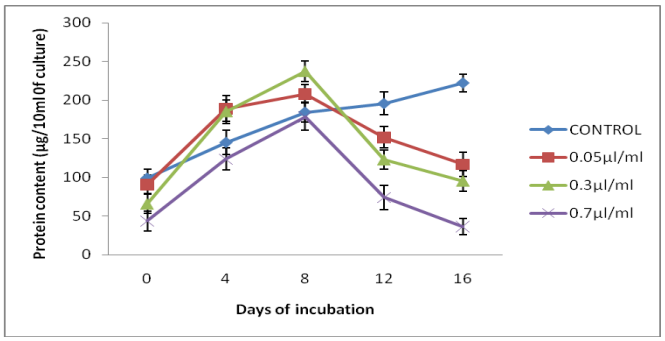

Figure 4d: Effect of various concentration of Chloropyriphos on the Protein content of *Anabaena variabilis*

4. Discussion

The indiscriminate use of insecticides on cyanobacterial population has been considered to be inhibitory at high doses [16]. The results obtained in the present investigation depicted a insecticide concentration and time dependent reduction in growth, pigment and protein content in both the species irrespective of their differential tolerance to a particular

concentration of the insecticide. In general, cyanobacterial interaction with toxic chemicals followed certain general trend such as partial inhibition, total inhibition, delayed inhibition, growth stimulation and growth resumption after a extended lag period. In certain cases the differential toxic effect was due to biodegradation, autodegradation, modification by organsm, decreased permeability of the chemical into the cell [17], [18], [19] .In the present investigation the delayed inhibitory effect of insecticides on both the test species may be attributed to the possible metabolization of the chemical or its degraded product by the cyanobacteria,since some species possess such a capacity [20], [21].

The present data obtained cleared a way that the use of high and continuous use of organophosphorus pesticide causes detrimental effect on rice field cyanobacteria. The growth in terms of chlorophyll-a was greatest in untreated cells, as compared to treated culture which might be due to inhibition on the photosynthetic activity of cyanobacteria by the continuous use of insecticides. Application of the test insecticides affected total carotenoids of *Anabaena variabilis* and *Nostoc commune*. The content of these pigments was decreased at 16days culture, at highest concentration of pesticides. The data obtained in the present paper reveals that protein content was decreased to a maximum at higher concentrations of both the insecticides. In conclusion it appears that both the strains of cyanobacteria in general do not resist to a very high concentration of insecticides Monocrotophos and Chloropyriphos. However the effect of pesticide on the population of nitrogen fixing cyanobacteria in rice fields also depends on other insecticide concentration and flooding of water associated with paddy fields. More detailed field studies are needed, avoiding the use of high application rates more than recommended will likely increase the more tolerant cyanobacteria.

References

[1] A.Watanabe, S. Nishigaki, and C. Konishi, " Effect of N2-fixing blue-green algae on the growth of rice plant." Nature, 168, 748–49, 1951.

[2] O.A. Herrero, A.M. Muro-Pastor and E. Flores "Nitrogen Controlling Cyanobacteria, J. Bacteriol., vol. 183, pp. 411-425, (2001)."

[3] U. Mishra and S. Pabby "Cyanobacteria: A Potential Biofertilizer for Rice," Res., 6-10, 2004.

[4] R.N. Padhy Cyanobacteria and Pesticides. Residue Reviews 95: pp.1-44, 1985.

[5] S. Singh , P. Datta and R. Patel " Survival and growth of diazotrophic cyanobacterial isolates exposed to rice field herbicides". Bulletin of Environmental Contamination and Toxicology 70: pp.1052-1058, 2003.

[6] C.R.M. Ravindran, S. Suguna, and S. Shanmugasundaram, "Tolerance of *Oscillatoria* isolates to agrochemicals and pyrethroid components". *Indian J. Exp. Biol.*, 38, pp. 402–404, 2000.

[7] N. Satish and G.L. Tiwari, " Pesticide tolerance in *Nostoc linckia* in relation to the growth and nitrogen fixation". *Proc. Natl. Acad. Sci. India*, 70, pp. 319–323, 2000.

[8] P.K. Shetty, J. Mitra, N.B.K. Murthy, K.K. Namitha, K.N. Savitha and K. Raghu, " Biodegradation of cyclodiene insecticide endosulfan by *Mucor thermohyalospora* MTCC 1384". *Curr. Sci.*, **79**, pp.1381–1383, 2000.

[9] F.B.C. Pankratz, C. Doebel, A. Farenhorst and L.G. Goldsborough, "Interactions between Algae (Selenastrum Capricornutum) and Pesticides: Implications for Managing Constructed Wetlands for Pesticides Removal," J. Env. Sci. Health: B., vol. 38, pp. 147-155, 2003.

[10] P.T.V. Lakshmi and Annamalai, "A Biochemical Studies on the Response of Organo-phosphorus Insecticide and Release of Extra Cellular Products by Cyanobacteria." Res Jrl of Fish and Hydrobiol, 2(1): pp.13-17, 2007.

[11] A.K. Ghadai, S. Kumar, and D.K. Acharya, "Bio-Molecular assay of cyanobacteria on response to diazinon an Organophosphorus insecticide"**.** Int Jr of Chem Res, I Volume 2, Issue 1, 2, pp-20-24, 2010.

[12] S.P. Adhikary, Growth measurement by maintaining light scattering of filamentous blue green algae which does not give uniform and stable suspenson in culture vessels zeit Allg Microbial 23: pp.475-483, 1983.

[13] G. Mackinney, Absorption of light by chlorophyll solutions. *Journal of Biology and Chemistry* 140: pp.315–322. 101: pp.107-116, 1941.

[14] B.H. Davis, Carotenoids in Chemistry and Biochemistry of plant pigment. Ed. Goodwin, T.W. Academic Press, New York and London, pp 149-155, 1976.

[15] O. Lowry, H. Rosenbrough, A. Farr, and R. Rondall, Protein measurement with the folin-phenol reagent.J.Biol.Biochem.,193:pp.265-275,1951.

[16] S. Panigrahi , S. Padhy , R.N. Padhy, " Toxicity of parathion-methyl to cells, akinetes and heterocysts of the cyanobacterium Cylindrospermum sp. and the probit analysis of toxicity". Annu. Appl. Biol. 143:pp.195–199, 2003.

[17] J. Thomas and S. Shanmugasundaram, "Interaction of agrochemicals with the cyanobacterium Anacystis nidulans and pattern of growth".Microbios letter,33:pp.115-120, 1986.

[18] H. Fang, Y. Yu, X. Chu, X. Wang, X. Yang, J. Yu, "Degradation of chlorpyrifos in laboratory soil and its impact on soil microbial functional diversity". J.Environ. Sci. 21, pp. 380–386, 2009.

[19] T.J. Emmerik, M.J. Angove, B.B. Johnson, J.D. Wells, " Sorption of chlorpyrifos to selected minerals and the effect of humic acid". J. Agric. Food Chem. 55 (18), pp.7527–7533, 2007.

[20] R. Arjmandi, M. Tavakol and M. Shayeghi, "Determination of Organophosphorus insectcide residues in the rice paddies," 2010.

[21] B.K. Singh, A. Walker and D.J Wright, "Bioremedial potential of fenamiphos and chlorpyrifos degrading isolates: influence of different environmental conditions.Soil Biol". Biochem. 38 (9), pp.2682–2693,2006.