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Estimating the Toxicity and Lethal Dose Concentration of Five Saline Salts and Two Pesticides to Cyanobacterium *Anabaena 7120*

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ABSTRACT

Cyanobacteria are considered as a helper microorganism in soil binding and soil formation in addition to incorporating nitrogen to the soil. They are adapted to a wide rage of habitats. Most cyanobacteria fix atmospheric nitrogen and supply to the plants. For this reason, the cyanobacteria are considered as one of the important groups of beneficial microbes. The present study aimed to study the influence of five saline salts and two insecticides on filamentous heterocystous spore forming, nitrogen-fixing cyanobacterium, *Anabaena 7120*. Except for NaCl and $MgCl_2$, other salts at lower concentrations (Na_2CO_3 at 40 mg, Na_2SO_4 at 100 mg and $MgSO_4$ at 50 mg) stimulated growth of *Anabaena 7120* as assessed by optical density and an increase in cell number. Higher concentrations of all salts retarded growth. Both pesticides at low concentrations (10 mg/l) enhanced the growth of the cyanobacterium.

Keywords: pesticide, salinity
Abbreviations: LD, lethal dose; **OD**, optical density; **PPM**, parts per million (equiv. to mg/l)

INTRODUCTION

Cyanobacteria are the most ancient oxygenic autotrophic organisms (Li *et al*. 2001). The cosmopolitan distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as heat, cold, desiccation, salinity and UV radiation (Gupta *et al*. 2008). Most cyanobacteria fix atmospheric nitrogen and supply to plants. For this reason, they are considered an important group of beneficial microbes. This organism enhances soil fertility in rice fields (Swaminathan 1984; Singh 1987). Cyanobacteria are believed to have originated three billion years ago and are considered as a good model system for studying plant responses to environmental stress (Castenbolz 1988). Salinity is one of the most important factors that limits the growth and productivity of plants, eukaryotic microorganisms and bacteria (Inabha *et al*. 2001). Applications of pesticides in agricultural fields to control harmful insects pests can cause harm or adversely affect non-targeted organisms like crop plants, microorganisms, etc. (Hill and Wright 1978). Pesticide concentrations in soil with inundated water are high enough to cause complete lethality to various microorganisms since they cannot act on them immediately but rather are themselves affected (Rajagopal *et al.* 1984).

Cyanobacteria are considered as one of the most beneficial microorganisms in soil involved in soil binding and soil formation in addition to fixing atmospheric nitrogen (Galhano *et al*. 2009). The negative impact of salt stress on various metabolic processes like photosynthesis, respiration and decrease in phycocyanins contents of cyanobacteria has been reviewed earlier (Sudhir and Murthy 2004). The available literature on cyanobacteria reveals the detrimental effects of pesticides and other toxic compounds on growth, CO_2 assimilation, O_2 evolution in photosynthesis, nitrogen fixation, mutagenesis and detoxification (Padhy 1985; Panigrahy *et al.* 2003). The present study aimed to study the influence of five saline salts and two commonly used insecticides on filamentous, heterocystous, spore-forming, nitrogen-fixing cyanobacterium, *Anabaena 7120*.

MATERIALS AND METHODS

Cyanobacterium strain

The cyanobacterium *Anabaena 7120* was obtained from Prof. L. A. Sherman, University of Missouri, Columbia and was maintained in the laboratory for using in the present study.

Salts and pesticides

Salts comprised carbonates, sulfates and chlorides of sodium and magnesium viz., NaCl, Na_2SO_4, Na_2CO_3, $MgSO_4$, and $MgCl_2$. All the salts were procured from Glaxo India Ltd., Mumbai, India. Chloropyriphos 20% EC, commercially known as pyrivol, is a broad-spectrum organophosphorus insecticide (Ralchem Ltd., Hyderabad, India). Monocrotophos, commercially known as sufos, is a systematic insecticide that contains 36% w/w monocrotophos as active ingredient (Sudarshan Chemicals, Pune, India).

The cyanobacterium was grown in batch culture in 50 ml culture tubes (200 × 20 mm) containing 10 ml of modified Chu-10 medium without nitrogen (Padhy and Singh 1978). Stock solution of pesticides and salts were filter sterilized and added aseptically in mg/l to the media after autoclaving at 121°C for 20 min at 1.06 kg cm^{-2}. Actively growing cyanobacterial stock of 0.5 ml with a mean cell density of 2.2×10^3 cells/ml (approx. $OD_{660\ nm} = 0.025$, estimated by a colorimeter Elico-100, ELICO, Hyderabad, India) was added to the growth media. The cultures were maintained at 28 ± 2°C under 24-h light at 2500 Lux. Growth of cyanobacterium was measured every 3 days. Mean cell number/filament were

Received: 26 October, 2008. Accepted: 25 August, 2009.

Research Note

determined for 20 random filaments using a light microscope (Carl Zeiss JENA, German DDR) with the aid of a haemocytometer (Neubauer, DDR Germany). Each experiment was repeated at least twice with three replicates. All the data were plotted as a graph using Origin 6.1 software.

To determine the lethal dose of salts and pesticides, cultures showing constant OD on respective days were pelleted at 5000 rpm for 10 min. The pellets were re-cultured in fresh medium to assess viability. If there was no visible growth after 20 days, the concentration of salt or pesticide was taken to be LC_{100}. LC_{100} is the minimum concentration of salt/pesticide at which the growth of the cyanobacterium completely ceases and cyanobacterium shows complete death.

RESULTS AND DISCUSSION

Salt stress induced a decrease in plant growth and reproductivity due to the disruption of physiological processes like photosynthesis by high salt concentrations (Sudhir and Murthy 2004). Salt stress mainly decreases the content of phycocyanins and there by interrupts the energy transfer from PBPS to PSII reaction center (Lu and Vonshak 2002; Allakhverdiev and Murata 2008). Sodium stress due to addition of NaCl, $NaNO_3$ and $NaHCO_3$ caused a reduction in energy transfer from allophycocyanin to PSII in *Spirulina pletensis* (Verma and Mohanty 2000). Moreover, salt stress cause a number of physiological changes like decrease in cytoplasmic volume, inactivation of sodium/hydrogen antiporters, electron transport system, etc. (Allakhverdiev and Murata 2008).

Effect of NaCl on the growth of cyanobacterium, *Anabaena 7120* is shown in **Fig. 1** and **2**. Maximum growth of the cyanobacterium was seen in plain Chu-10 medium without NaCl, increasing up to the 12th day. NaCl at all concentrations (2500–30000 mg/l) had varying growth-retarding effects. The lethal dose (LC_{100}) of NaCl for the cyanobacterium was 30000 mg/l. NaCl in trace amounts is essential for some metabolic functions in cyanobacteria but its elevated level (200 mM) might inhibit growth (Bhadauriya *et al.* 2007).

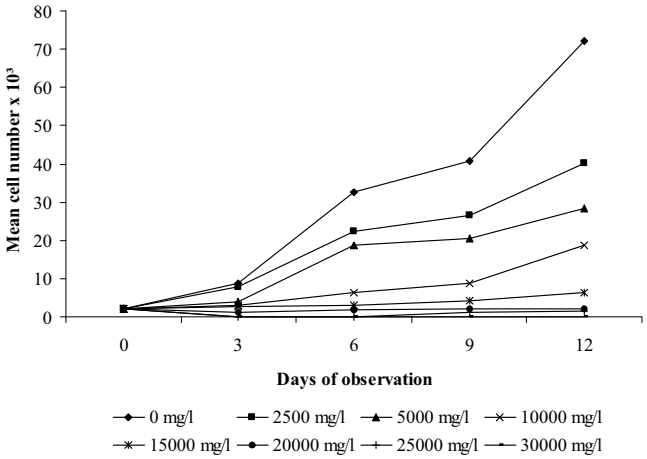

Fig. 1 Effect of NaCl on mean cell number of *Anabaena 7120.*

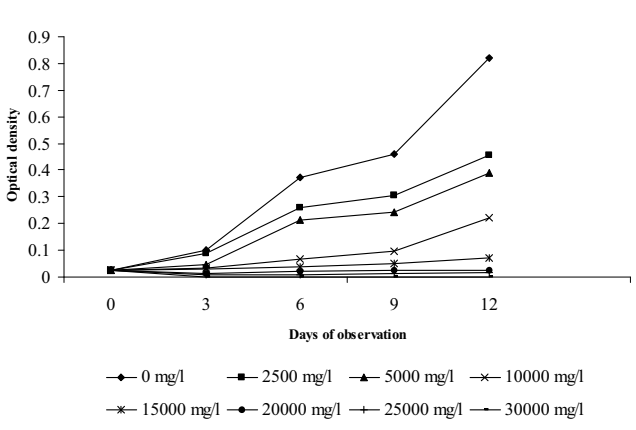

Fig. 2 Effect of NaCl on O.D of cultured *Anabaena 7120.*

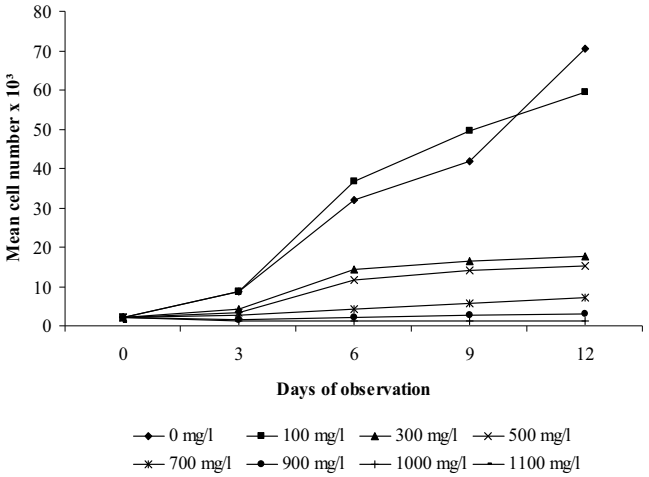

Fig. 3 Effect of Na_2SO_4 on mean cell number of *Anabaena 7120*.

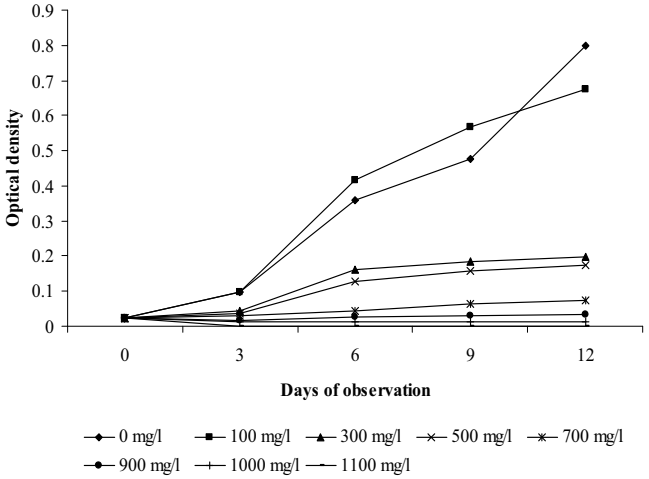

Fig. 4 Effect of Na_2SO_4 O.D of cultured *Anabaena 7120.*

The influence of Na_2SO_4 on the growth of the cyanobacterium is shown in **Fig. 3** and **4**. A stimulatory effect of Na_2SO_4 on the growth of the cyanobacteria at 100 mg/l was observed on the 6th and 9th days (cell number and OD, respectively) but showed a decline in growth on the 12th day. All other concentrations showed a declining trend in growth pattern of the cyanobacterium. From the results, it was found that the LC_{100} of Na_2SO_4 was 1100 mg/l.

As Na_2CO_3 is a media component in Chu-10 medium (20 mg/l), the effect of this salt was studied by adding multiple amounts of the original concentration into the medium (**Figs. 5, 6**). Concentration of 40 mg Na_2CO_3 stimulated the growth of the cyanobacterium as observed on 3rd, 6th and 9th day but later (12th day) it slightly retarded growth. The LC_{100} of Na_2CO_3 was 600 mg/l.

The growth-inhibiting effect of $MgCl_2$ on cyanobacterium was pronounced at all the concentrations tested, i.e. 200-1500 mg/l (**Figs. 7, 8**). Medium without $MgCl_2$ showed maximum growth. The LC_{100} of $MgCl_2$ was 1500 mg/l.

Like Na_2SO_4, $MgSO_4$ is also a media component of Chu-10 medium. The concentration of $MgSO_4$ in Chu-10 medium is 25 mg/l. The effects of $MgSO_4$ were similar to Na_2SO_4. The influence of $MgSO_4$ on growth of cyanobacterium in terms of OD and cell number is presented in **Fig. 9** and **10**. 50 mg/l stimulated growth throughout the experimental period while 100 mg/l stimulated the growth of cyanobacterium up to the 9th day. The LC_{100} of $MgSO_4$ was 800 mg/l.

The detrimental effects of NaCl to cyanobacterium on various physiological processes have been extensively stu-

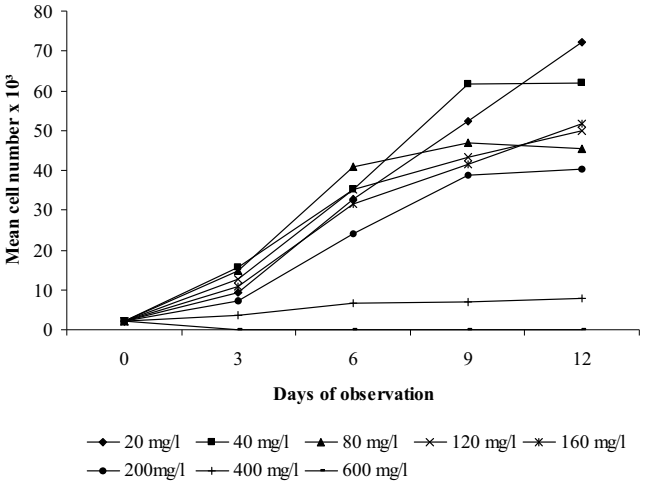

Fig. 5 Effect of Na_2CO_3 on mean cell number of *Anabaena 7120*.

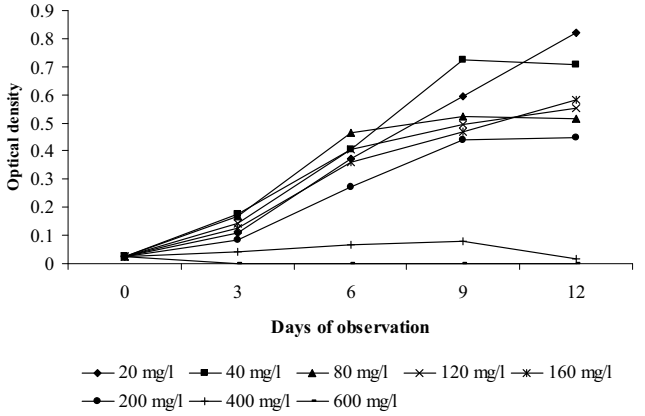

Fig. 6 Effect of Na_2CO_3 on O.D of cultured *Anabaena 7120*.

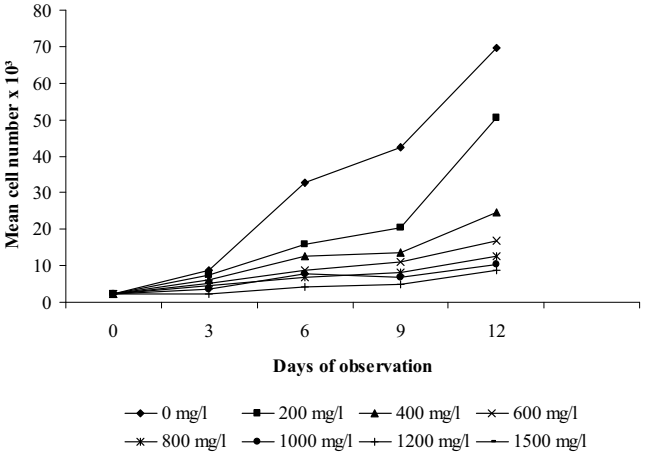

Fig. 7 Effect of $MgCl_2$ on mean cell number of *Anabaena 7120*.

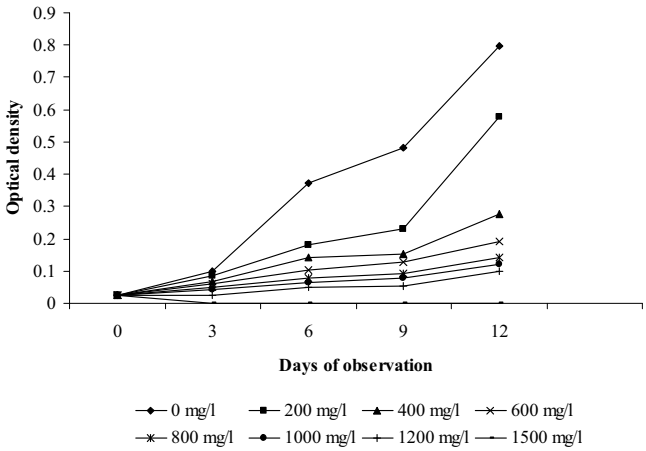

Fig. 8 Effect of $MgCl_2$ on O.D of cultured *Anabaena 7120*.

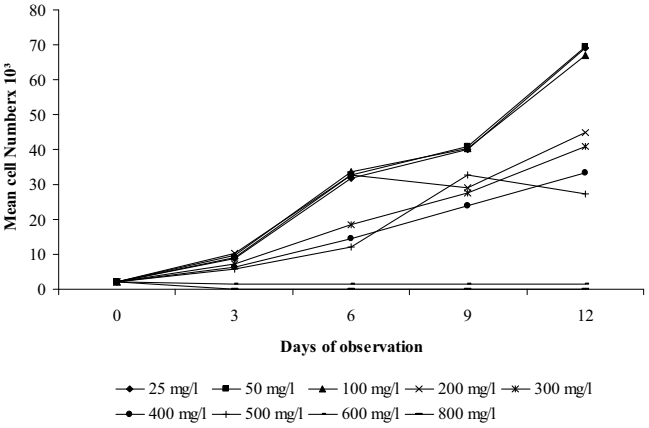

Fig. 9 Effect of $MgSO_4$ on mean cell number of *Anabaena 7120*.

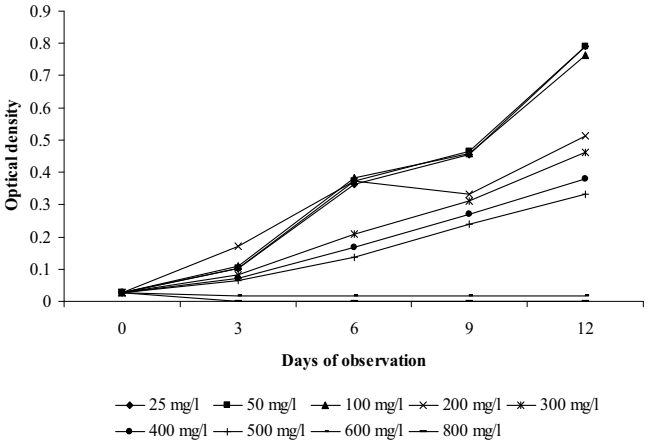

Fig. 10 Effect of $MgSO_4$ on O.D of cultured *Anabaena 7120*.

died (Hagermann and Erdmann 1997; Padhi *et al.* 1997/98; Zeng *et al*. 1998; Lu and Vonshak 2002; Bhadauriya *et al.* 2009) although all other saline salts (e.g. Na_2SO_4, Na_2CO_3, $MgCl_2$, $MgSO_4$) have not. Salt stress in the natural environment is caused not only by NaCl but also by the combination of other salts. Limited studies on effect of other salts on cyanobacteria have been conducted *in vitro* (Torrecilla *et al*. 2001; Ning *et al*. 2002)

The influence of monocrotophos on *Anabaena* growth in Chu-10 medium is shown in **Fig. 11** and **12**. At 10 mg/l, monocrotophos enhanced the growth of the cyanobacterium although a steady decline in growth was noticed at all other concentrations tested. The LC_{100} of monocrotophos was 200 mg/l. Pesticides, in addition to controlling pests, are harmful to a wide variety of beneficial microorganisms as they persist in the ecosystem for a longer duration (Padhy 1985; Kiran *et al*. 2006).

Chlorpyriphos showed a stimulatory effect on the growth of cyanobacterium at 10 mg/l up to the 9th day. On 12th day, there was a decrease in growth (**Figs. 13, 14**) for all the concentrations tested. The LC_{100} was 120 mg/l.

When observed under light microscope, there were broken and fragmented cells of cyanobacterium seen at LC_{100} concentrations of all the saline salts and pesticides (photo not shown).

Cyanobacteria are quite sensitive to herbicides because they share many common characteristics with higher plants and green algae. This sensitivity of cyanobacteria towards herbicides varies but depends mainly on species and kind of herbicide (Leganés and Fernández-Valiente 1992). Panigrahy and Padhy (2000) clearly showed that two insecti-

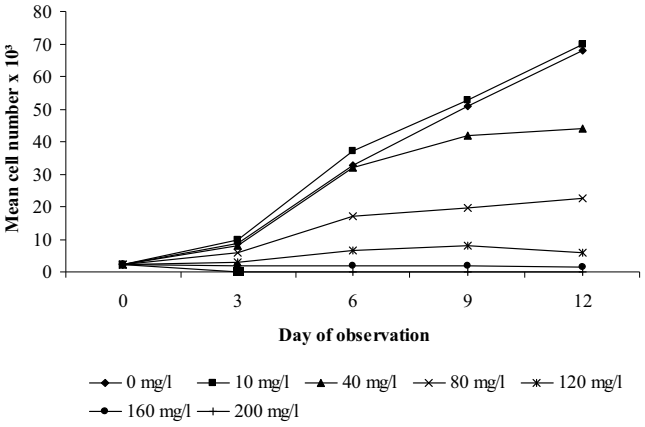

Fig. 11 Effect of monocrotophos on mean cell number of *Anabaena 7120.*

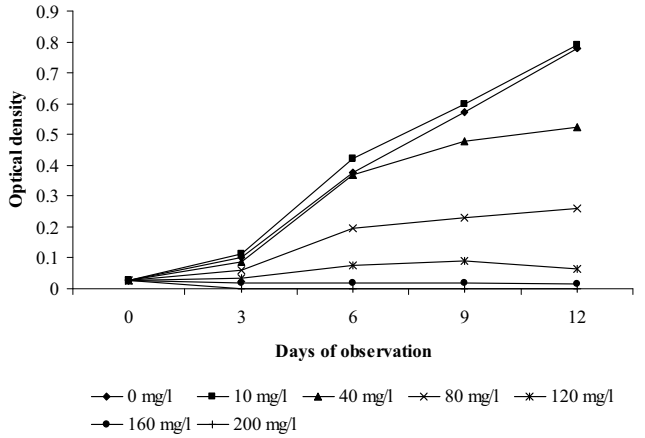

Fig. 12 Effect of monocrotophos on O.D of cultured *Anabaena 7120.*

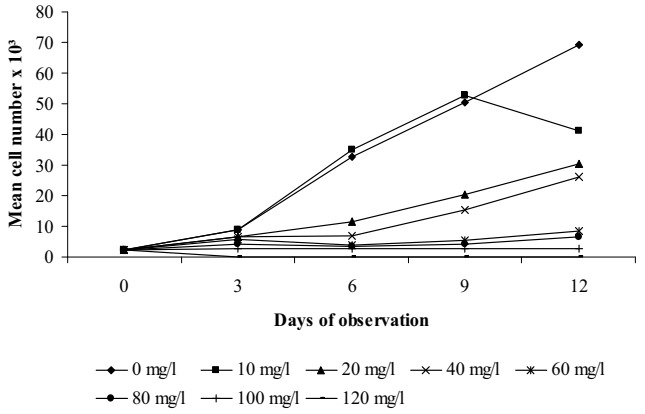

Fig. 13 Effect of chloropyriphos on mean cell number of *Anabaena 7120.*

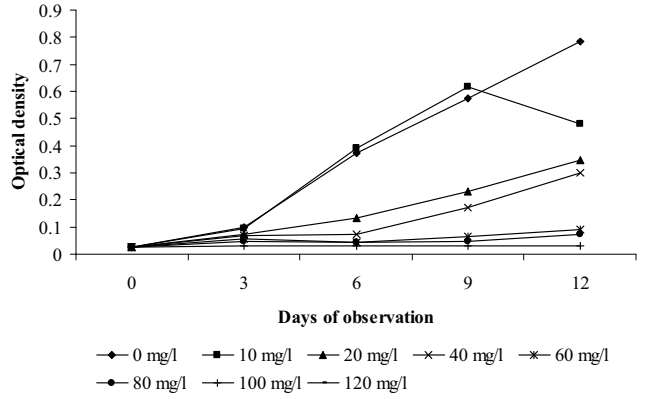

Fig. 14 Effect of chloropyriphos on O.D of cultured *Anabaena 7120.*

cides and five fungicides caused instant cell death of a cyanobacterium, *Cylindrospermum* sp. At lower concentrations of pesticides, there was a stimulatory effect on growth of cyanobacterium in the present study. As observed in our studies, the effect of pesticides on cyanobacterial population has been considered stimulatory at lower concentrations and inhibitory at higher doses (Panigrahi *et al.* 2003; Kiran *et al.* 2006; Rajendran *et al*. 2007). The stimulatory effect may be due to the degradation of the pesticides by blue-green algae. Biodegradation of organophosphorous pesticides by cyanobacteria has been reported (Caceres *et al*. 2008).

Among the two pesticides studied, chloropyriphos was more harmful to the cyanobacterium, *Anabaena 7120*, than monocrotophos.

These are some initial findings that show the lethal dose concentration of five salts and two pesticides. There is a need to do further studies at the physiological and molecular levels in this regard and with other blue-green algae to create mutants and to apply biotechnological tools for genetic modification to tolerate salinity and pesticide stress.

ACKNOWLEDGEMENTS

Authors thanks Mr. Nageswar Rao and Mr. S. N. Rao, CSMCRI (Bhavnagar) local field station at Berhampur, for their support and encouragement to carry out major part of the present study. Authors also thank Dr. Urmil J. Mehta and Dr. A. Nookaraju for their support and cooperation during preparation of the manuscript.

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