International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 256-267**
http://www.ijcmas.com

Original Research Article

Isolation and identification of cynobacteria (*Oscillatoria pseudogeminata* G.schmid) from marine water and its potential on remediation of pesticide

R.Bakiyaraj[1], L.Baskaran[1] and T.Senthilkumar[2]*

[1]Department of Botany, Annamalai University Annamalai Nagar – 608 002, Tamil Nadu, India
[2]Department of Botany, Govt Arts College (Autonomous), Salem, Tamil Nadu, India
**Corresponding author*

A B S T R A C T

The present study was conducted in the laboratory to test the effect of carbaryl on growth and biochemical components. Growth was measured in terms of chlorophyll 'a' content and it was decreased in all the concentrations 100,200,300,400,500 and 1000 ppm of pesticide. Carotenoids and phycobilins were significantly reduced. Carbohydrate, protein, amino acid contents were also decreased gradually with increasing concentration of pesticides. Lipid level was decreased drastically in all treated concentration except control. Further research on this organism with different herbicides, pesticides and fungicides is needed to consider the usage of this organism as a successful biofertilizer.

Keywords

Pesticide; carotenoids; phycobilins; carbohydrate; protein; amino acid.

Introduction

Cyanobacteria are oxygenic photosynthetic prokaryotes and widely distributed in the natural ecosystems such as freshwater and seawater. The sources of water are rainwater, backwater, surface water and seawater. The rainwater carries the washed out minerals, salts and organic substances from the earth's surface and is stored in ponds, lakes and rivers. Sea water is alkaline because of high salt content. Natural water contains numerous organisms like blue green algae, phytoplanktons, zooplanktons, and fish. Natural and synthetic chemical compounds called pesticides hold a very important place in agriculture and economics. Pests like various algae, weeds, fish, nematodes, fungi, bacteria and insects can have a devastating effect on crop yield either by direct destruction of the crop or by competing for nutrients in the soil. Pesticides are used globally and extensively for the control of such pests. Satifactory crop yield is impossible without the use of pesticides despite the public awareness that now exists about harmful effects of pesticide use. Besides agriculture, pesticides are widely used in industrial, domestic and marine environments. Pollution of natural waters particularly fresh and sea water implies that it contains lot of inorganic and organic substances introduced by human activities which change its quality and are harmful to many living organisms, including man.

Water pollution is now one of the most serious problem in the world. Water that flow on the surface of crop fields, where agro chemicals such as fertilizers, pesticides, herbicides and insecticides are used contribute a lot to water and soil pollution. Pesticides may enter water supplies from several sources. These include careless over spraying of water courses, drains or standing water run-off from sprayed or treated areas, careless disposal of containers, washing down of contaminated equipments and leaching from soil in treated areas. The application of pesticides to areas adjacent to streams and rivers frequently lead to contamination of these aquatic habitats. Sea and ocean are the ultimate destination for most of the end-products of manmade pollution. However, sea and ocean from the massive sources of primary production of organic matter by planktonic algae, the starting point of all natural aquatic food chain.

Pesticides enter the environment by direct and indirect routes. These agents can be sprayed on crops, dispersed in the field as a granular substance or dispersed from aircraft as in agriculture. Such pesticides as algicides are introduced directly into the aquatic environment. The cleaning of industrial mixing equipment, disposal of waste, and accidents increase the amount of pesticides found in soil and water. Leaching, runoff and aerial drift account for pesticide residues in water that was not initially applied in an aquatic habitat. The aquatic biotopes include the lowly organized primitive plant like bacteria, fungi, algae and the most advanced ones, the flowering plants. Among algae, the blue-green algae are widely distributed algal organisms in clean and polluted waters of lakes, ponds and reservoirs, in fresh and salt water. The blue green algae form a significant part of the free-floating population of microscopic organisms which constitute the plankton of eutrophic lakes and oceans. Pesticides introduced into the aquatic systems may cause stresses and deleterious effects on the micro algae especially blue green algae. Some of these agrochemicals such as pesticides remain unchanged for a very long time in the environment. These are not easily degraded in the natural environment. They get incorporated into the food chain and get accumulated in algal cells. Such non-degradable pollutant not only accumulate but are often "biologically magnified" as they more in biogeochemical cycles along food chain. Also they frequently combine with other compounds to produce additional toxins.

Such cyanobacteria are also employed to monitor pesticide pollution as it posse's major threat to the marine environment in recent years. The occurrence of pesticides in the marine environments (Selvaraj *et al*., 1985) prompted the present investigation to study the probable effects of the pesticide carbaryl on the marine cyanobacterium *Oscillatoria pseudo geminata* occurring very commonly in South East coast of India.

Materials and Methods

Samples collection

Samples were collected from Naga pattinam coastal area located on the South East coast of India (Latitude 10^0 49^0 N, Longitude 79^0 43^0 E) and transported to the laboratory for the isolation of the test organism. The samples were spread in petridish with streak agar plates. After 7 days the unialgal cultures were isolated. At this stage microscopic examination was made to identify that the unialgae isolated were single species. The organism thus

chosen was the cyanobacterium *Oscillatoria pseudogeminata* G.Schmid. The above organism was identified with the help of standard works of Desikachary (1959).

Culture medium

The culture medium used in the present study was ASN-III-N^- medium. The composition used for preparing the medium was described by Waterbury (1976) and Rippka *et al* (1979).

Description about *Oscillatoria pseudogeminata*

Oscillatoria pseudogeminata marine algae collected from Nagapattinam Ditstrict, is a single filamentous among the other algae. Thallus pale or dirty blue-green, trichomes coiled, pale blue-green, ends not attenuated, 1.3 – 2.2 µ broad; cells as long as broad or somewhat longer or shorter than broad, about 2.6 µ long, or not constricted at the cross-walls. Cross-walls thick, not granulated, end cell rounded, calyptra absent (Plate-I).

Effect of pesticide carbaryl

To test the effect of pesticide on growth of *Oscillatoria pseudogeminata* was treated with different concentration of pesticide which was used as carbaryl. The insecticide used was of high quality carbaryl (Sevin) manufactured by Bayer Crop science Limited, Gujarat.

Stock solution

Prepared by dissolving in 1 g carbaryl in 100ml of sterile media = 10,000 ppm

Stock solution of the pesticide carbaryl was prepared in sterile media (ASN-III-N^- medium) from which different concentrations of Carbaryl (ppm) were prepared *viz*. 100,200,300,400,500 and 1000 ppm respectively (PLATE-II).

Exponentially growing *Oscillatoria pseudogeminata* was inoculated into 100 ml of each test solution taken in 250 ml conical flasks and experiments were conducted. This experiment was conducted for 24 days in a culture room illuminated with white fluorescent light (2,000 lux) by maintaining a 10/14 light dark cycle at 22 ± 1^0C. Duplicates were run in all the experiments. The test organism treated with different concentrations of Carbaryl is observed under microscope and care was taken to record the morphological characteristics including abnormalities.

Growth measurements

Growth rate was measured in terms of chlorophyll-a as biomass components (McKinney's method, 1941) at initial (0) day 4th, 8th, 12th, 16th, 20th and 24th days after inoculation.

All the growth analysis *viz.,* carotenoids, phycobilins, carbohydrates, proteins, total lipid and free amino acids content calculated at 24st day after the inoculation. Estimation of carotenoids followed by Davis, 1976, 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

Effect of different concentrations of

pesticide carbaryl on the growth, carotenoids, phycobilin pigments and biochemical constituents such as carbohydrate, protein, lipid and amino acid of *Oscillatoria pseudogeminata* are shown in Figure 1 to 7.

Growth was measured in terms of chlorophyll 'a' in all the seven treatments. In control, growth was well pronounced up to 12 days from the day of inoculation, Then there was a gradual decline in growth till the end of experimental period (Fig.1). In other treatments (100,200,300,400,500 and 1000 ppm) there was sight enhancement up to 4 days followed by a lag phase of another 4 days (8th day). Then gradual increase up to the 12th day and showed a gradually decrease up to 24th day. Maximum growth was observed in control and minimum was noticed at 1000 ppm on the 12th day.

As seen in chlorophyll 'a' in control, carotenoid pigments were also found to be steeprise upto 4days, then there was a slight increase up to 12th day followed by a steady decline in growth tiil/the end of the experimental period (Fig.2). In control, maximum carotenoid content (0.078 µg/ml) was noticed in 12th day and minimum (0.049 µg/ml) on 24th day. In all other concentrations there was a gradual increase in carotenoid pigment upto 12th day followed by a gradual decline up to 24th day. Drastic reduction was observed at 1000 ppm throughout the experimental period when compared to control.

Among the phycophilins, allophycocyanin and C-phycocyanin pigments were recorded in all the concentrations including control. Phycoerythrin wasn't observed in the test organism. *Oscillatoria pseudogeminata* of the two pigments allophycocyanin was found to more than phycocyanin at 100, 200 and 500 ppm including control. Whereas at 300, 400 and 1000 ppm. Phycocyanin was more than allophycocyanin. Maximum reduction of allophycocyanin and C-phycocyanin were 88% and 86% respectively at 1000 ppm and minimum (24% and 17%) at 100 ppm were observed (Fig. 3).

Proximate comparison (carbohydrate, protein, lipid and amino acid) of *Oscillatoria pseudogeminata* estimated at the different concentration of pesticide Carbaryl is shown in figure 4 to 7. There was a greater reduction in the level of carbohydrate (44%) noticed at the highest concentration (1000 ppm) of pesticide on the 24th day and the lesser reduction (14%) at 100 ppm as compared to control (Fig.4) as the concentration of pesticide increases, the total carbohydrate level decreased considerably.

As can be seen from the protein content was maximum (37µg/ml) in control, Apart from control, all the concentration showed gradual reduction in protein content. Nearly 40% reduction of protein was observed in *Oscillatoria pseudogeminata* treated with 1000 ppm, but in the lowest concentration (100 ppm) only 11% reduction was noticed as compared to control (Fig. 5).

Lipid level was decreased drastically in treated increasing pesticide concentration except control. The highest reduction of 79% and the lowest of 12% of lipids were observed at 1000 ppm and 100 ppm respectively (Fig.6). With regard to the amino acid content (Fig. 7). The maximum reduction (71%) was found at 1000 ppm

PLATE – I

The test alga *Oscillatoria pseudogeminata* G.Schmid

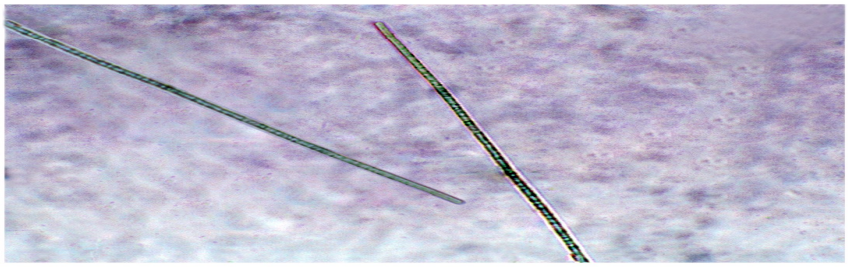


PLATE- II

Different treatment with pesticide on *Oscillatoria pseudogeminata*

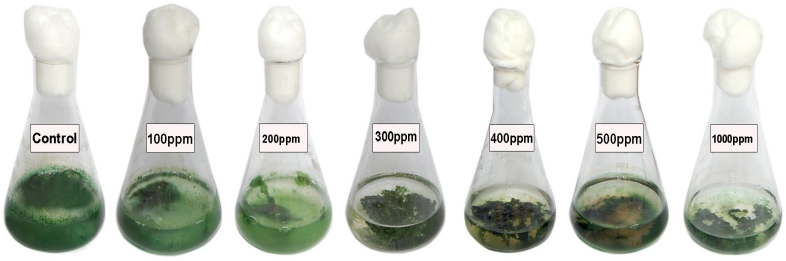
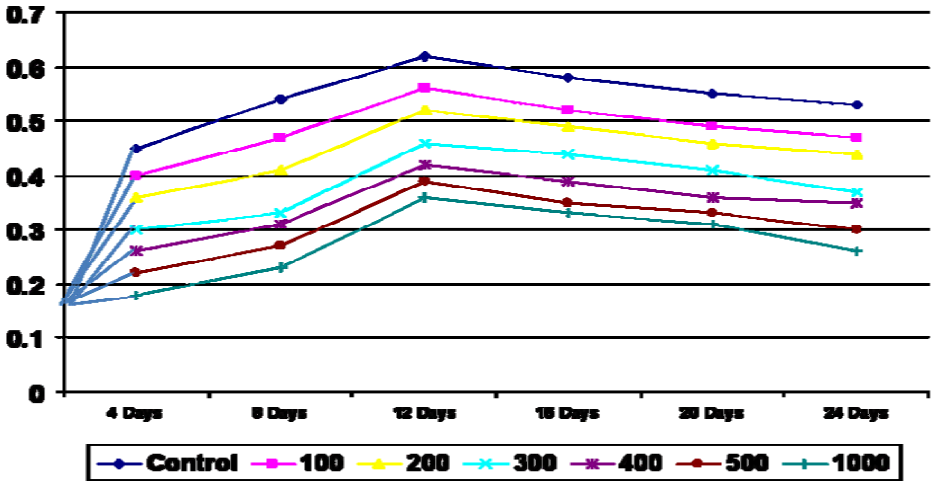


Fig.1 Effect of different concentration of pesticide Carbaryl on Chlorophyll 'a' content of *O. pseudogeminata*



o

Concentration in ppm

Fig.2 Effect of different concentration of pesticide Carbaryl on Carotenoids content *O. pseudogeminata*

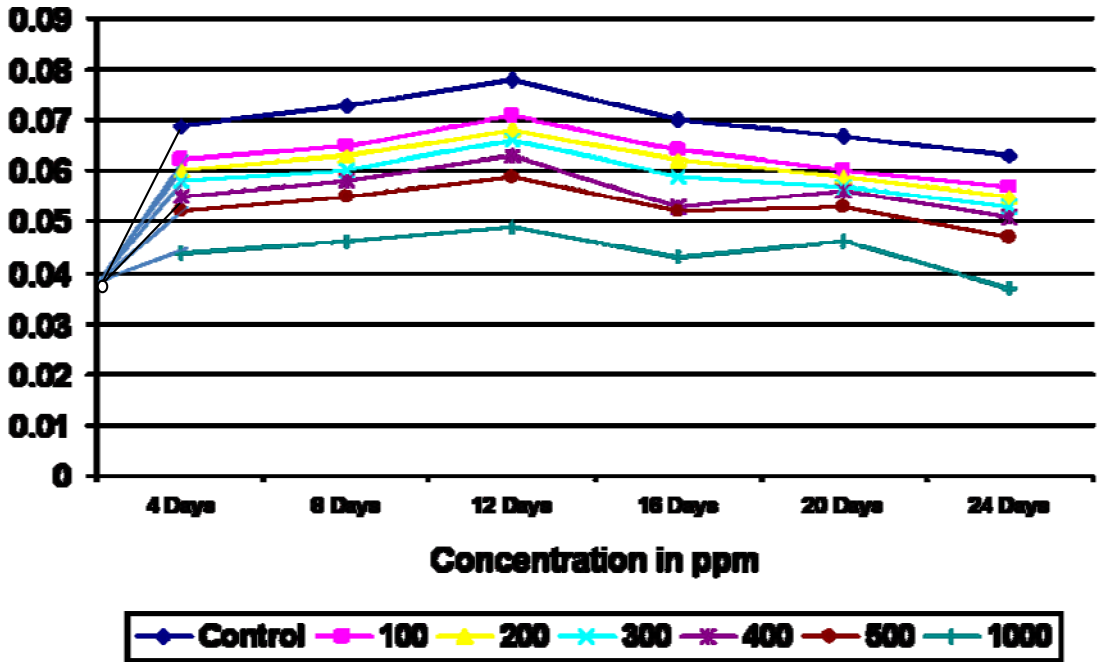


Fig.3 Effect of different concentration of pesticide Carbaryl on Phycobiliproteins of *O. pseudogeminata*

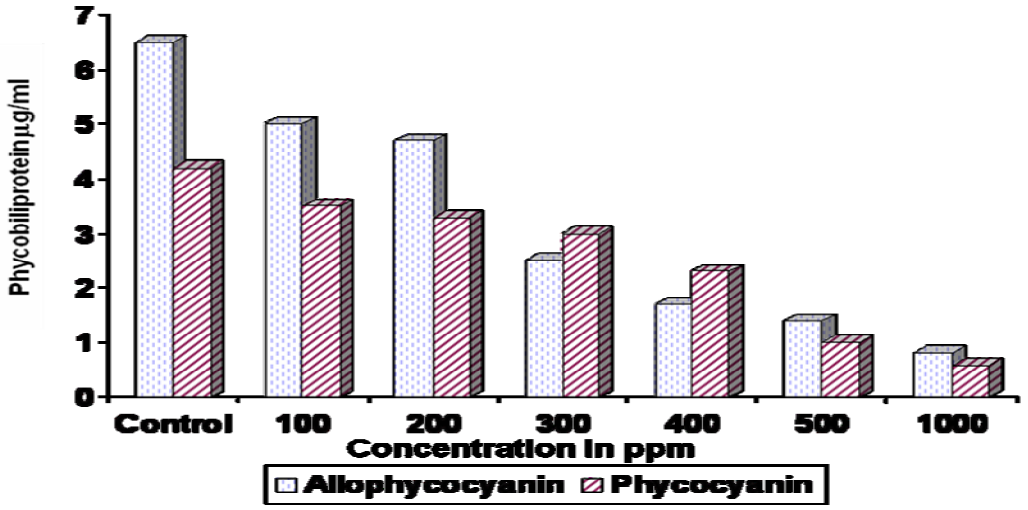


Fig.4 Effect of different concentration of pesticide Carbaryl on Carbohydrate of *O. pseudogeminata*

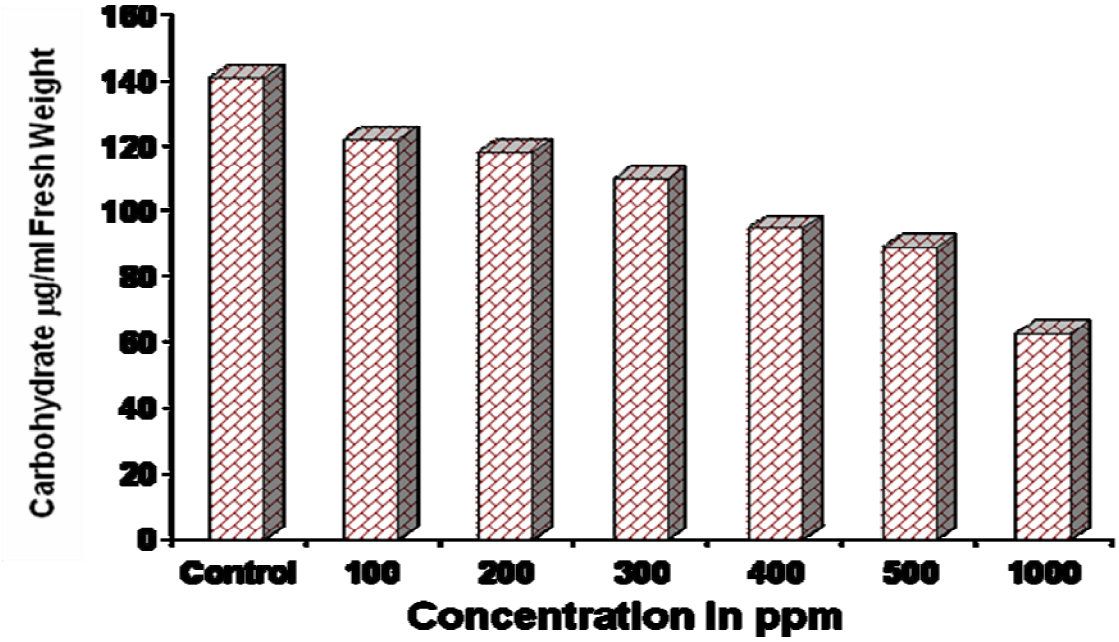


Fig.5 Effect of different concentration of pesticide Carbaryl on Protein of *O. pseudogeminata*

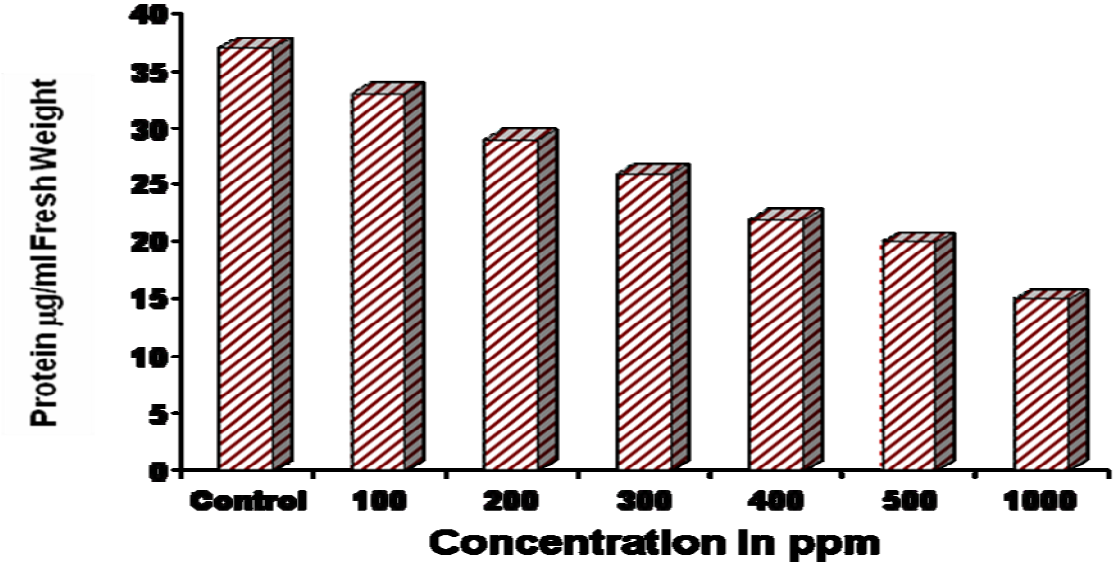


Fig.6 Effect of different concentration of pesticide Carbaryl on Lipid content of *O. pseudogeminata*

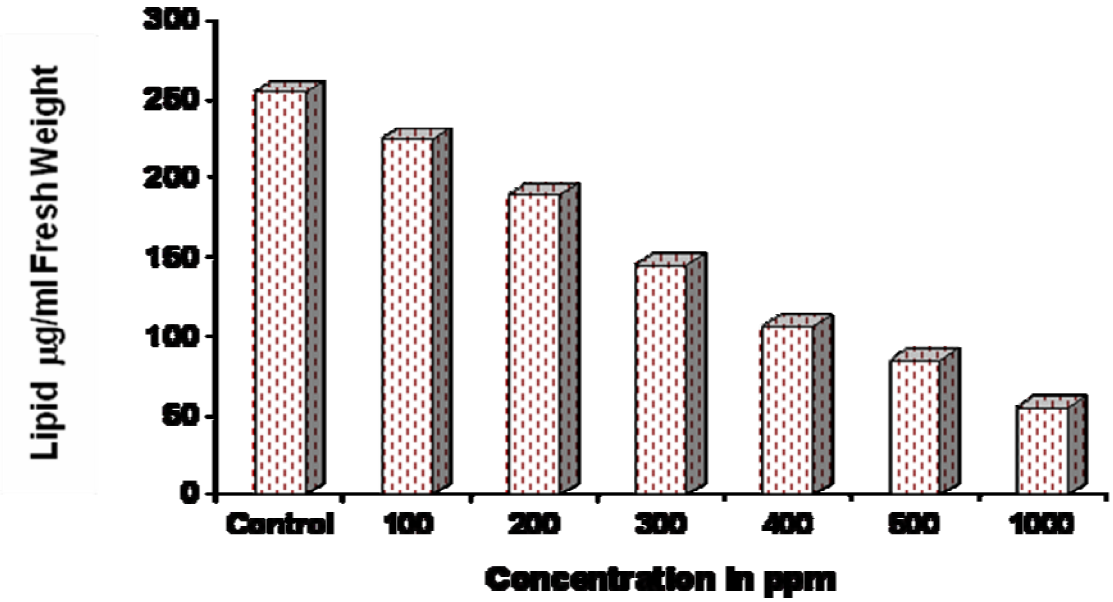
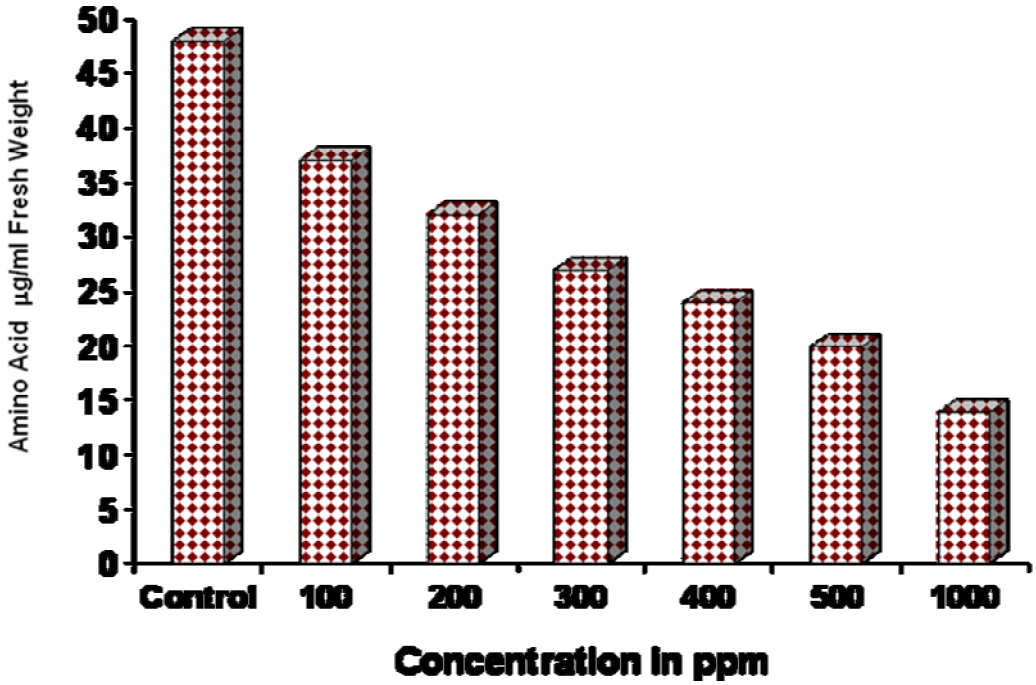


Fig.7 Effect of different concentration of pesticide Carbaryl on Amino acid content of *O. pseudogeminata*



and minimum (22%) at 100 ppm were also decreased gradually with increasing concentration of pesticide.

In the present study, the growth of cyanobacterium *Oscillatoria pseudo geminata* was to be influenced by the pesticide carbaryl at the different concentration *viz*. 100, 200, 300, 400 and 1000 ppm. Agrochemicals that are used in rice cultivation include basically two groups of chemical substances namely pesticides and fertilizers. Pesticides are either organic or inorganic chemicals mainly used for pest control. In a highly concentrated state these compounds are very toxic to all plant systems. Besides killing all the target organisms when applied excessively, they inhibit the growth of other non-target organisms (Cullimore and McCann, 1977; Stration and Corke, 1979). Many workers have also reported the effect of pesticides on the tolerance, growth and nitrogen fixation of blue green algae (Venkataraman and Rajalakshmi, 1971; Rath and Mishra, 1977; Patnaik and Singh, 1978; Sahu *et al.*, 1992) which form an interesting group, as they are abundant in soil, paddy fields and other habitals where they enhance the maximum nitrogen fixation (Das Silva *et al.*, 1975; Huang, 1978).

In the present study, pesticide carbaryl was found to inhibit the growth of *Oscillatoria pseudogeminata* upto 8 days at all concentration tested compared to control, but there was a slight increase in growth upto the 8 day, followed by sudden increase upto the 12th day. Then suddenly decline in growth afterwards. It may be concluded that pesticide carbaryl at higher concentration adversely affects the growth and cell multiplication of *Oscillatoria pseudogeminata***.** Similar observations were reported in *Anabaena* CH2 and CH3 treated with herbicides Butachlor and Benthicarb where the increased concentration of herbicides decreased the level of chlorophyll, nitrogen fixation, photosynthetic and respiratory activities (Chen, 1986).

Muruganantham and Manoharan (1998) observed the inhibition of chlorophyll 'a' in *Westiellopsis prolifica* treated with different concentrations of 2, 4-D. Similar type of differential effect of various pesticdes on growth and nitrogen fixation of different algae has earlier been reported (Da Silva *et al.*, 1975; Kapoor and Sharma, 1980; Sardeshpande and Goyal, 1982). It is further evident from the morphological observations that the filaments of *Hapalosiphon welwitchii* exposed to pesticide showed many small gas vacuoles within the cells. Similar observations were made by Selvaraj *et al.* (1985) who reported small translucent bodies in marine diatom *Pleurosigma elongatum* exposed to pesticde. Lindane and this could be attributed due to the synthesis or accumlation of some metabolic products as a result of stress at 1000 ppm the filaments were pale green in colour, which indicates that this concentration would be lethal to test alga.

Influence of pesticide on the carotenoid and phycobilin pigments of the test alga was similar to that observed for growth. This in in agreement with the findings of Kobbia *et al.*, (1991) who has investigated the effects of pesticde trifluralin suppressed the total carotenoid accumlation in *Anabena and Nastoc***.** Similarly the repressed effect of phycobilin pigments has been reported by Chen (1986) who observed the algae *Anabaena* exposed to Butachlor, reduced 51% allophycocyanin and 58% of C-phycocyanin and Bromocil at 50 ppm

inhibited 25% of allophycocyanin and 45% C-phycocyanin pigments.

The carbohydrate, protein and lipid contents were decreased with increased concentration of pesticide. The present findings agree well with those of Megharaj *et al*. (1991) who proved that pesticide exposed algae *Nostac Linckia* and *N. muscorum* inhibited the total carbohydrate level even at the lowest concentration. Similar other observations were also made by many workers (Anand and Veerappan, 1980; Shabana, 1985; Mansour *et al.*, 1994). Likewise *Westiellopsis prolific* treated with 2,4-D showed maximum reduction of carbohydrate, protein and lipid content in all concentration (Muruganantham and Manoharan, 1998). The report made earlier by Kashyap and Pandey (1982), Kent and Currie (1995) also confirm this.

The amino acid content of the test algae was decreased at the increased concentration of pesticide. Similar increase as also been reported by Mishra and Nanda (1997) who found that the free amino acid content decreased significantly with the increasing concentrations of mercury contaminated soils. Muruganantham (2001) also observed the decrease in amino acid content with increasing concentration of heavy metal copper. In contrast, Khalil (1992) investigated that the amino acid content stimulated on *Phormidium fragile* exposed to lindane.

From the foregoing discussion, it is clear that studies pertaining to the effect of pesticide could influence the cyanobacterium *Oscillatoria pseudogeminata* by reducing its growth and biochemical constituents. Algae have tremendous capacity to absorb and accumulate pesticide residues in their cells which is an essential feature used for decontamination of pesticides in aquatic system. Further research in essential to findout the indicator algae for removing the pesticide from the aquatic environment.

The present study "Effect of pesticide Carbaryl on the Cyanobacterium *Oscillatoria pseudogeminata"* was conducted in the laboratory to test the effect of carbaryl on growth and biochemical components. Growth was measured in terms of chlorophyll 'a' content and it was decreased in all the concentrations (100,200,300,400,500 and 1000 ppm) of pesticide. Carotenoids and phycobilins were significantly reduced. Carbohydrate, protein, amino acids contents were also decreased gradually with increasing concentration of pesticides. Lipid level was decreased drastically in all treated concentration except control. Further research on this organism with different herbicides, pesticides and fungicides is needed to consider and use this organism as a successful feed and biofertilizer in future.

References

Anand, N. and Veerappan, B., 1980. Effect of pesticides and fungicide on blue green algae. *J.Phykos.,* 19(2); 210-212.

Chen, P.C., 1986. Effects of herbicides on growth and photosynthesis of *Anabaena CH2 and CH3. Proc. Natl. Sci.Counc. B.Roc., 10(3); 151-156.*

Cullimore, D. and McCann, A., 1977. Influence of four herbicides on the algae flora of prairie soil. Plant soil, 46; 499-510.

Da Silva, E.J., Henriksson, L.E. and

Henriksson, E., 1975. Effect of pesticides on blue green algae and nitrogen fixation. *Arch. Environ. Contami, Toxicol*., 3; 193-204.

Davis, B.H., 1976. Carotenoids. In: Chemistry and Biochemistry of plant pigments, T.W. Goodwin (ed.), 2:38-165.

Desikachary, T.V., 1959. Cyanophyta, Indian Council of Agricultural Research, New Delhi.

Dubois, M., Gilles, R.A., Hamilton. F.K., Robers, P.A. and Smith, F., 1956. Calorimetic method for determination of sugar and related substances, *Anal. Chem., 28:350-336*.

Huang, C.Y., 1978. Effects of nitrogen fixing activity of blue-green algae on the yield of rice plants. *Bot. Bull. Academic, Sinica, 19: 41-52.*

Jayaraman, J., 1981. Calorimetric estimation of amino acids. In: Laboratory Manual in Biochemistry, Wiley Eastern Ltd., New Delhi, p.64.

Kapoor, K. and Sharma, V.K., 1980. Effect of certain herbicides on survival, growth and nitrogen fixation of blue green alga *Anabaena doliolum* Bharadwaja *Zeits. Allgemeine Microbio.*, 20(7): 465-469.

Kashyap. A.K. and Pandey, K.D., 1982. Inhibitory effects of rice-field herbicide machete on *Anabaena doliolum Zpflanzcenphysiol. Bd.*, 107(5): 339.

Kent, R.A. and Currie, D., 1995. Predicting algal sensitivity to a pesticide stress. *Environ. Toxical. Chemis.*, 14(6): 983-991.

Khalil.Z.1992. Interactions of pesticides with freshwater algae. *Isotope and Radi. Res.*, 24(1): 61-69.

Kobbia, I.A., Shabana, E.F., khalil, Z. and Zaki, F.T., 1991. Growth criteria of two common cyanobacteria isolated from Egyptian flooded soil, as influenced by some pesticides. Water, Air and Soil pollu., 60(1&2): 107-116.

Lowery, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.j., 1951. Protein measurement with the Folin-phenol reagent. *J. Bio. Chem.*, 193: 269-275.

Mansour, F.A., Abdel-Rahman, L., Soliman, S.A., Shaaban-Desouki and Hussein, M.H., 1994. Effect of herbicides on cyanobacteria changes in carbohydrate content, P Mase and got activities in *Nostoc kihlmani* and *Anabaena oscillarioides.* J. Phykos 33 (1&2): 153-162.

McKinney, G., 1941. Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, 140; 315-322.

Megharaj, M., Pearson, H.W and Venkateswarlu, K., 1991. Toxicity of p-aminophenol and p-nitrophenol to *Chlorella vulgaris and two* species of *Nostoc* isolated from soil. *Pesti. Biochem. Physio.*, 40(3): 266-273.

Mishra, B.B. and Nanda, D.R., 1997. Reclamination with cyanobacteria. Toxic effect of mercury contaminated waste soil on biochemical variables. *Cytobios.*, 92(370-371): 203-208.

Muruganantham, A. and Manoharan, C., 1998. Effect of 2,4- Dichlorophenoxy acetic acid on *Westiellopsis prolifica* Janaet. Seaweed Res. Utiln., 20(1&2): 97-102.

Muruganantham, A., 2001. Effect of heavy metals on the cyanobacterium, *Hapalosiphon welwitschii*, W. et. G.S. West. M.Phil. Thesis, Bharathidasan University, Tiruchirapalli, pp.37.

Patnaik, U. and Singh, P.K., 1978. Effect of nitrate nitrogen on the growth heterocyst differentiation and nitrogen fixation in rice field blue-green alga

Gloeotrichia sp. *Arch. Hydrobiol Supp. Algological Stud.*, 51; 318-327.

Rath, S.C. and Mishra, B.N., 1977. Effect of Dimecron-100 an organophosphorous insecticide on growth tolerance and nitrogen fixation of *Westiellopsis prolifica* Janet. Comp. Physiol. Ecol., 2: 162-167.

Rippka, R, Derulles,J, Waterburry, J.B., Hardman, M. and Stanier, R.Y., 1979. Genetic assignments strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol* 111: 1-61.

Sahu, J., Das, M.L. and Adhikary, S.P., 1992. Reaction of blue-green algae of rice field soils to pesticide application. *Trop. Agric. (Trinidad), 69: 362-364.*

Sardseshpande, J.S. and Goyal. S.K., 1982. Effect of insecticides on growth and nitrogen fixation by blue-green algae. *Proc. Natl. Symp. BNF*, New Delhi, pp. 586-605.

Sato, N. and Murata, M., 1988. Membrane Lipids. In: Methods of Enzymology (eds.) Packer, L. and A.N. Glazer, 167: 251-259.

Selvaraj, C., Jayachandran, S and Kannan, L., 1985. Studies on the effect of an organochlorine pesticide on *Pleurosigma elongatum* W.Smith. Isolated from the Pichavaram Mangrove Area. *The Mangroves: Proc. Nat. Symp. Biol. Util. Cons. Mangroves*, pp 378-381.

Shabana, E.F., 1985. Use of algal bach assays to assess the toxicity of atrazine to some selected cyanobacteria. 1. Influence of atrazine on the growth pigmentation and carbohydrate contents of *Aulosira fertilissima, Anabaena oryzae, Nostoc muscorum* and *Tolypothrix tenuis. Egypt. J. Physical. Sci., 12(1): 67-76.*

Stration, G.W. and Corke, C.T., 1979. The effect of nickel on the growth, photosynthesis and nitrogenase activity of *Anabaena inaequalis*. *Co*n. J. Microbio, 25: 1094-1099.

Venkataraman, G.S. and Rajalakshmi, B., 1971. Tolerance of blue-green algae to pesticides. *Curr. Sci.*, 40: 143.

Waterbury, J.B., 1976. Purtification, development patterns and properties of some freshwater and marine cyanobacteria belonging to the orders Chamaesiphonales and Pleurocapsales, Ph.D. Thesis, Univ. of California, Berkeley.