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Original Research Article

Pigment Analysis of a Blue-Green Alga and Use of Pesticide: A Proportional Perspective

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Abstract

The article takes into account the extraction of different types of pigments of a blue-green alga, *Anabaena cylindrica* Lemm. with a pesticide, Sevin (50% W.D.P. based on carbaryl-1-naphthylmethyl carbamate), under laboratory conditions. In order to know the extent of toxicity five different concentrations of Sevin were taken – LC_0 (2.13 ml l^{-1}), LC_{10} (2.54 ml l^{-1}), LC_{50} (3.01 ml l^{-1}), LC_{90} (3.25 ml l^{-1}), LC_{100} (3.35 ml l^{-1}). Uni-algal, axenic culture of *A. cylindrica* was inoculated and the survival percentage was determined. Three lethal concentrations (LC_{10}, LC_{50} and LC_{90}) were chosen to study the differential effects of different concentrations of Sevin on *A. cylindrica*. Observations (in 15 days duration) were made on how chlorophyll, carotenoid and pheophytin contents of *A. cylindrica* decreases with increasing concentration of toxicants resulting in a significant decline in the photosynthesis rate of exposed seedlings. Drastic depletion in chlorophyll content in exposed cultures only indicated either the destruction of the chlorophyll molecule or non-synthesis of chlorophyll in exposed algal cultures. The phaeophytin content declined significantly and a maximum of 87.91% decrease was recorded on 15th day of exposure. Further depletion up to 97.35% was recorded during recovery studies. Significant depletion in carotenoid content in exposed cultures to the tune of 92.4% on 15th day of exposure and further depletion by 98.9% on 15th day of recovery and its non-recovery during recovery period indicates total destruction of the pigment. The pigment ratio value in LC_{10} was much higher than the control organism. It is observed that from the data on toxicology of the present pesticide. Sevin can be a useful insecticide if used in the recommended dose and it can be intelligently used to have harmless effects in comparison to other pesticides available in the market. Farmers need to be exposed to these scientific findings to get the ultimate result of the use and abuse of the insecticide Sevin in order to reduce its adverse effects on the gross ecological imbalance, and the revival of the crop friendly alga for increasing crop productivity that the human beings and the animal world consume daily.

Keywords

Agro-chemicals
Anabaena cylindrica
Pesticides
Photosynthesis
Pigment analysis
Toxicant

Introduction

Pesticides have been increasingly used now–a-days to meet daily needs of human beings irrespective of their adverse effects on health and hygiene. Pesticides or agro-chemicals are chemicals designed to combat the attacks of various pests on agricultural and horticultural crops. They fall into three major classes: insecticides, fungicides and herbicides (for weed killers). There are also rodenticides (for control of vertebrate pests), nematicides (to kill microscopic eelworms), molluscicides (to kill slugs and snails) and acaricides (to kill mites). Insecticides are of 6 types: organochlorine insecticides, organophosphate insecticides, carbamates, pyrethroid, sulfonylurea and bio-pesticides. Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not the case, so the controversy of use and abuse of pesticides has surfaced. The rampant use of these chemicals, under the adage, "if little is good, a lot more will be better" has played havoc with human and other life forms (Aktar et al., 2009). The present study has been carried out to analyze the pigment contents of a blue-green alga, *Anabaena cylindrica* Lemm. in presence of a pesticide, Sevin (50% W.D.P. based on carbaryl-1-naphthylmethyl carbamate), under laboratory conditions.

Materials and methods

Test organism

Anabaena cylindrica Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. The spores and vegetative cells are always cylindrical in shape. The vegetative cells fix CO_2 and evolve O_2 where as heterocysts are unable to fix CO_2 or evolve O_2 but can fix nitrogen under aerobic condition (Stewart, 1974). The akinetes are perennating spores that develop between vegetative cells and heterocysts and obtain fixed carbon and nitrogen from them.

Selection of toxicant

SEVIN 50% W.D.P. based on carbaryl-1-naphthylmethyl carbamate, a broad spectrum pesticide for control of pests on fruits, vegetables, forage, cotton and other crops, as well as poultry and pets was selected for the present study. It is relatively free from handling hazards and may be applied in the immediate pre-harvest period without concern for excessive residues. SEVIN 50% W.D.P. has a low mammalian toxicity. It is generally regarded as one of the safer insecticides Sevin 50% W.D.P. is normally non-phytotoxic Sevin 50% W.D.P. is compatible with most of the pesticides, except those of alkaline nature.

Selection of concentration of pesticide and duration: The selected concentrations were 2.13 ml/L,2.54ml/L, 3.01 ml/L,3.25 ml/L ,3.35ml/L and exposure were 0,3,6.9,12 and 15 days. After exposure the alga were allowed to recover in normal condition in three consecutive periods of 5 days up to 15 days.

Pigment analysis-Photosynthetic efficiency

(a) Pigment studies (Estimation of chlorophyll, carotenoid and phaeophytin): For chlorophyll estimation, a culture suspension (100 ml) was withdrawn aseptically after a desired time interval (3 days) and centrifuged. The supernatant was discarded and the residue was extracted in 5 ml of 80% acetone for 10 min in dark and then kept in refrigerator (20°C) for 25 h. It was finally centrifuged to obtain a clear supernatant. The total chlorophyll was measured (Vernon, 1960) by recording the optical density of the extract at 649, 652 and 665 nm and for carotenoid at 475 nm against acetone reference in a UV- visible spectrophotometer (PC based) (Systronics, 119). The supernatant thus obtained was taken and a pinch of oxalic acid was added and kept for 24 h in a refrigerator for phaeophytin extraction (Vernon, 1960). The total phaeophytin was measured by recording the optical density of the extract at 536, 655 and 666 nm. The amount of total chlorophyll and phaeophytin was calculated by using the formula given by Vernon (1960). The amount of carotenoid was calculated by using the formula given by Davies (1976).

Carotenoid mg/litre = DVK/2000

Where, D=Optical density; V=Volume; K=Dilution factor.

The amount of total chlorophyll and phaeophytin was calculated by using the formula given below:

Total chlorophyll (mg l^{-1}) =
(6.45×OD at 665 nm) + (17.22×OD at 649 nm)

Total phaeophytin (mg l^{-1}) =
(6.75×OD at 666 nm) + (26.03×OD at 655 nm)

Statistical analysis

The data was processed initially for determination of mean values and standard deviation. Correlation coefficient, regression analysis and analysis of Variance Ratio (ANOVA) tests were conducted to analyze the obtained data (Panigrahi and Sahu, 2000).

Results

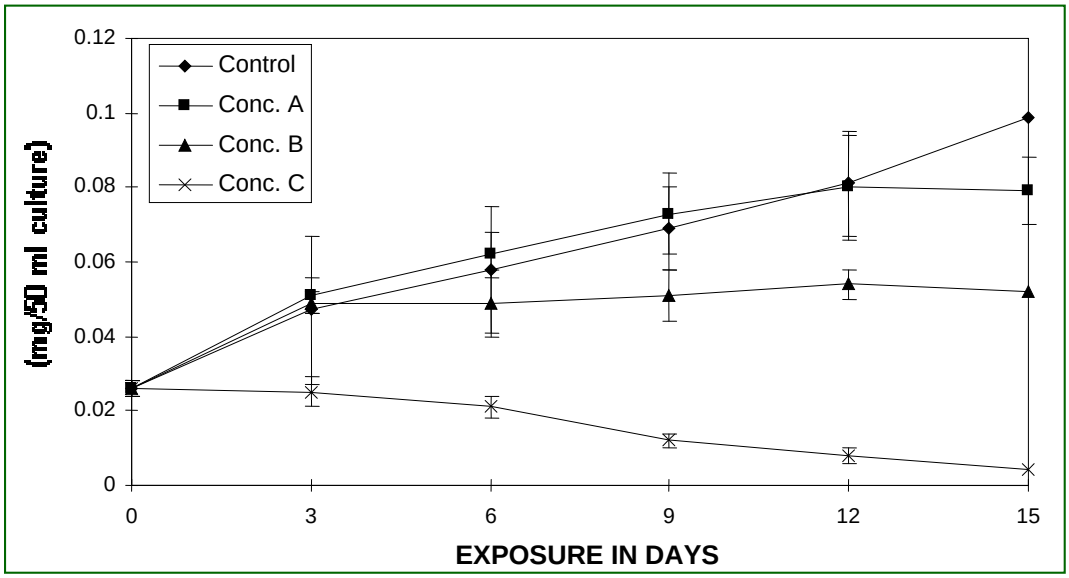
A graded series of concentrations of the pesticide, Sevin was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. Unialgal, axenic culture of *A. cylindrica* was inoculated and the survival percentage was determined. Table 1 describes the toxicity values of the pesticide, Sevin on *A. cylindrica.* The following concentrations were selected for further detailed studies pertaining to the effects of Sevin on the blue green alga.

Table 1. Deduced lethal concentration values after 15 days of exposure from the toxicity testing data.

Lethal Concentration (LC)	Pesticide Concentration (ml l^{-1})	Percent Survival (PS)
LC_0	2.13	PS_{100}
LC_{10} (A)	2.54	PS_{90}
LC_{50} (B)	3.01	PS_{50}
LC_{90} (C)	3.25	PS_{10}
LC_{100}	3.35	PS_0

In all figures, LC_{10}, LC_{50} and LC_{90} were expressed as A, B and C respectively and 'Con.' stands for control. Table 1 indicated that with the increase in concentration of the toxicant (Sevin) the survival percent decreased significantly showing a negative correlation. The above three lethal concentrations were chosen to study the differential effects of different concentrations of the pesticide (Sevin) on the blue-green alga, *A. cylindrica*.

Fig. 1: Changes in total chlorophyll content (mg/50 ml culture) in control and Sevin exposed *Anabaena cylindrica* at different exposure periods.



The changes in total chlorophyll content of *A. cylindrica* exposed to different sub-lethal concentrations of the pesticide, Sevin, at different days of exposure and recovery were shown in Figs. 1 and 2. Control set showed a regular trend of increase in chlorophyll content with the exposure period. The total chlorophyll content increased from 0.026±0.002 to 0.099±0.014 mg/50ml culture within 15 days of exposure and the pigment further increased to 0.131±0.008 mg/50 ml culture in the next 15 days of recovery. However, at LC_{10} (2.5 ml l^{-1}) a non-significant increase in chlorophyll content was marked over the control value up to 9th day of exposure and then the value declined with the increase in exposure period. The chlorophyll content increased during the recovery period but the values were less than the control values (Fig. 3). At 3.0 ml l^{-1} (LC_{50}) of Sevin chlorophyll content depleted significantly when compared to control values at all exposure periods, except third day of exposure, where 4.2% increase was recorded. However, the change in chlorophyll content with the increase in exposure period showed a positive correlation. With the increase in exposure period, the chlorophyll content increased. In contrast, at 3.25 ml l^{-1} (LC_{90}) after exposure, the chlorophyll content reduced drastically, even the values were much less than the inoculated value of '0' day of exposure. When the exposed alga was transferred to toxicant free nutrient medium, the exposed alga (C) could not recover altogether to its control value.

Fig. 2: Percent change in total chlorophyll content in Sevin exposed *Anabaena cylindrica*, in comparison to respective control values, at different exposure periods.

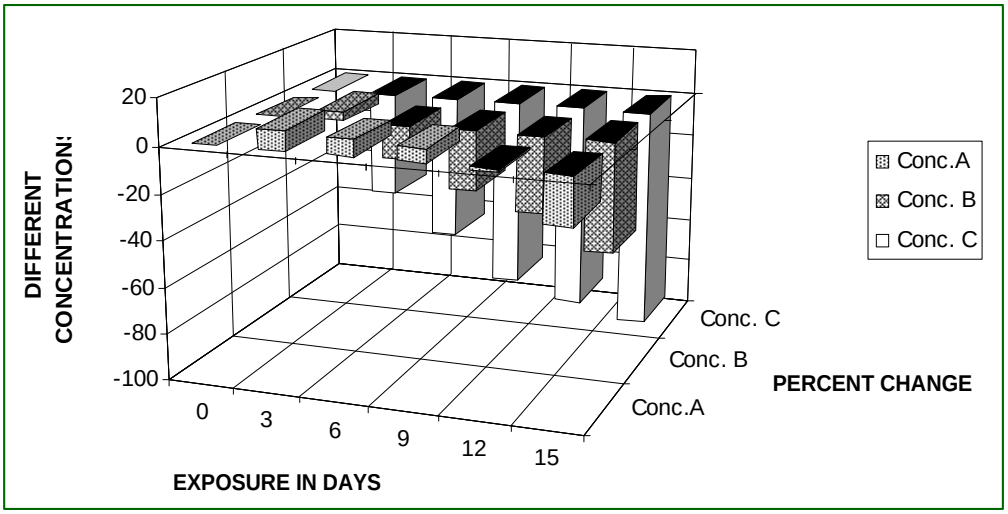
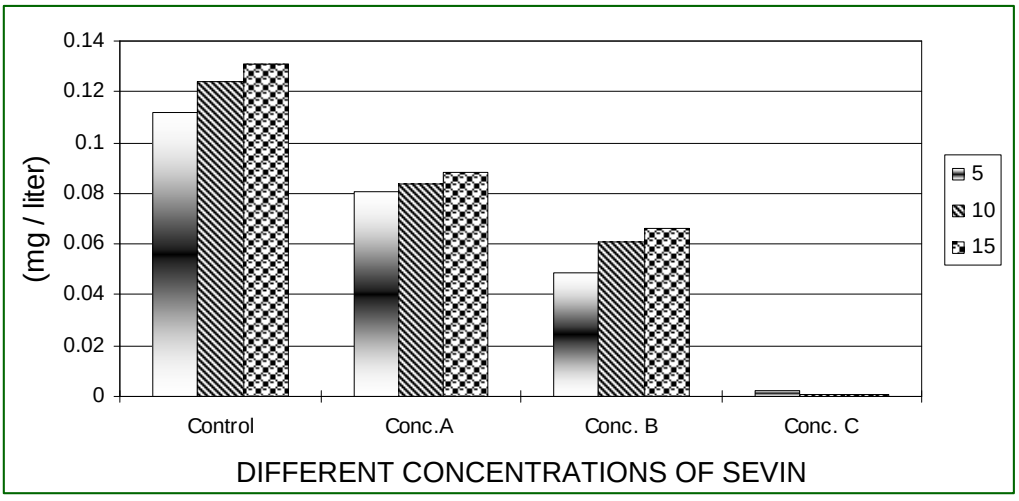


Fig. 3: Changes in total chlorophyll content in Sevin exposed *Anabaena cylindrica* at different recovery periods.



Changes in the total phaeophytin content of *A. cylindrica* after exposure to different concentrations of pesticide, Sevin, at different days of exposure and recovery has been presented in Fig. 4. Control set showed a positive correlation with the increase in exposure period and showed a steady increase after the very first day of inoculation for the entire period of exposure. Identical increase in phaeophytin content was marked in case of LC_{10} (A) and LC_{50} (B) of Sevin, whereas C (LC_{90}) showed drastic decline in phaeophytin content at all exposure periods. In all the exposed flasks, decline in phaeophytin content was observed when compared to the control values. No significant recovery was marked, when the exposed algae were transferred to toxicant free nutrient medium, except in conc. A, where a maximum of 7.39% recovery, when compared to 15d exposure value and no recovery at higher concentrations of the pesticide was marked (Figs. 5 and 6).

Fig. 4: Changes in total phaeophytin content in control and Sevin exposed *Anabaena cylindrica* at different exposure periods.

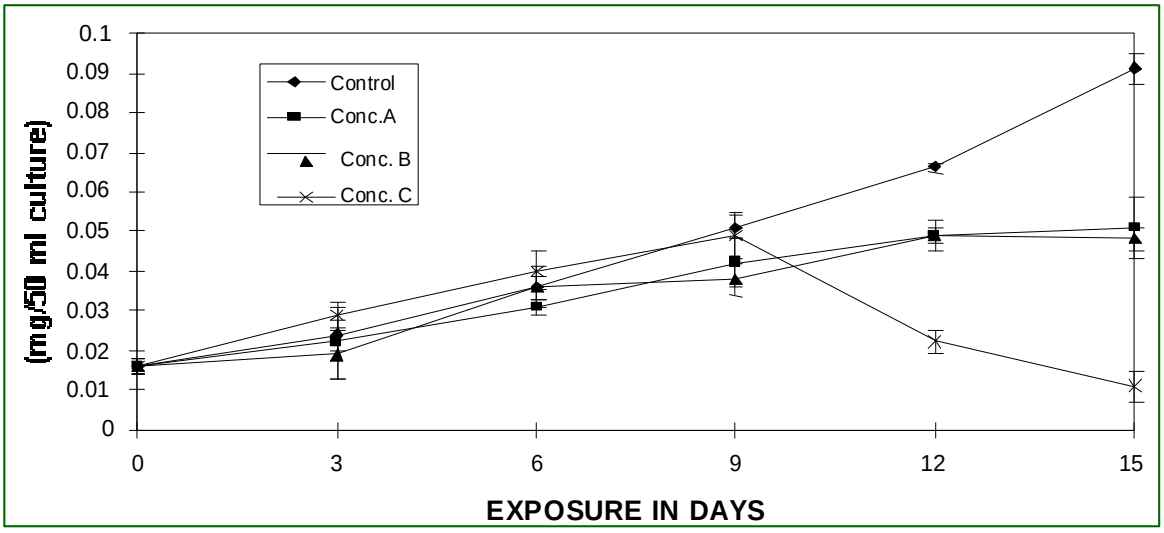


Fig. 5: Percent change in phaeophytin content of exposed *Anabaena cylindrica* in comparison to respective control values, at different exposure periods.

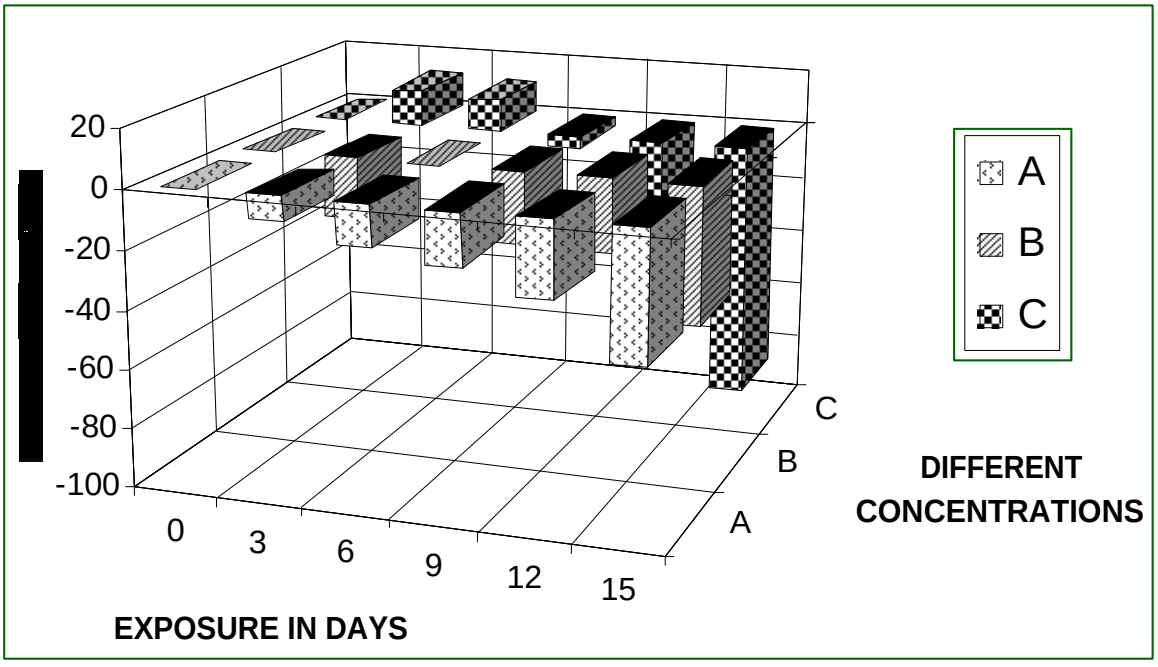
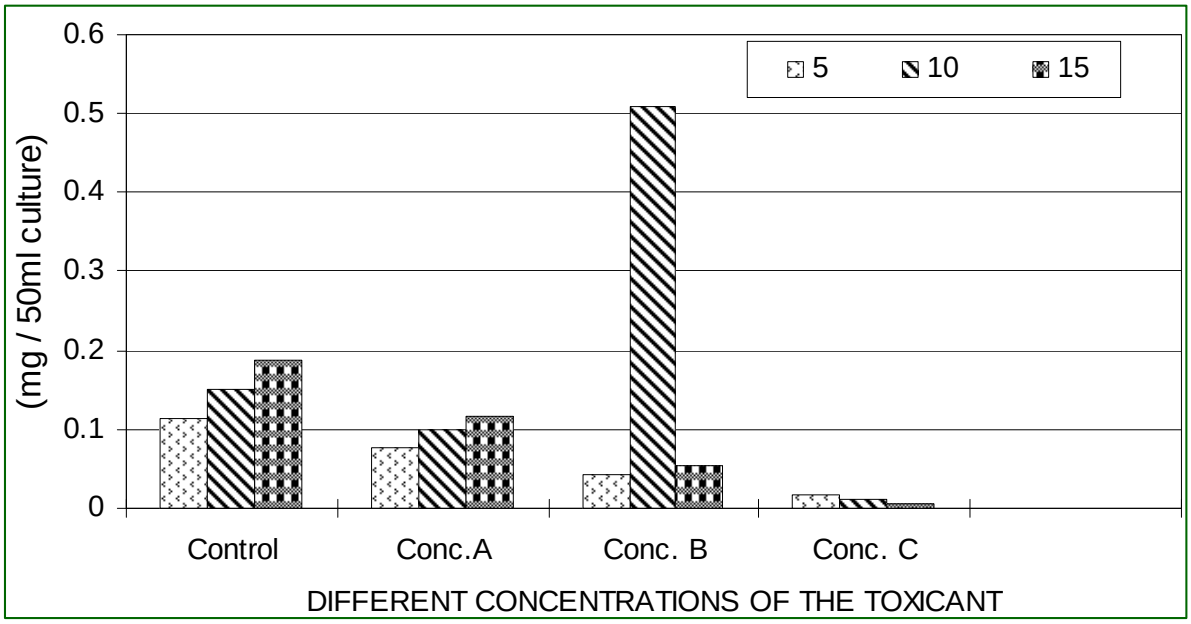


Fig. 6: Changes in total phaeophytin content in control and Sevin exposed *Anabaena cylindrica* at different recovery periods.



Figs. 7 and 8 represented the changes in the carotenoid content of *Anabaena cylindrica*, after exposure to different concentrations of Sevin at different days of exposure and recovery. Control set showed a steady increase in carotenoid content with the increase in exposure period, showing a positive ($r = 0.986$, $p \leq 0.001$) correlation. However, at all the three different concentrations of Sevin, a significant decrease in carotenoid content was marked (Fig. 7 and 8), when compared to their respective control values. At higher concentration (3.25 ml l^{-1}) of Sevin, a drastic decline in the pigment content was marked, when compared to the respective control values. Non-significant (Table 2) partial recovery in the rate over the 15th day value was marked in A & B, whereas in conc. C, no recovery was marked (Fig.9), rather the values were much less when compared to inoculation day value (Fig. 9). showed higher percentage of decrease in carotenoid content in exposed alga at all the three concentrations of Sevin.

Fig. 7: Changes in carotenoid content (mg/50 ml culture) in control and Sevin exposed *Anabaena cylindrica* at different exposure periods.

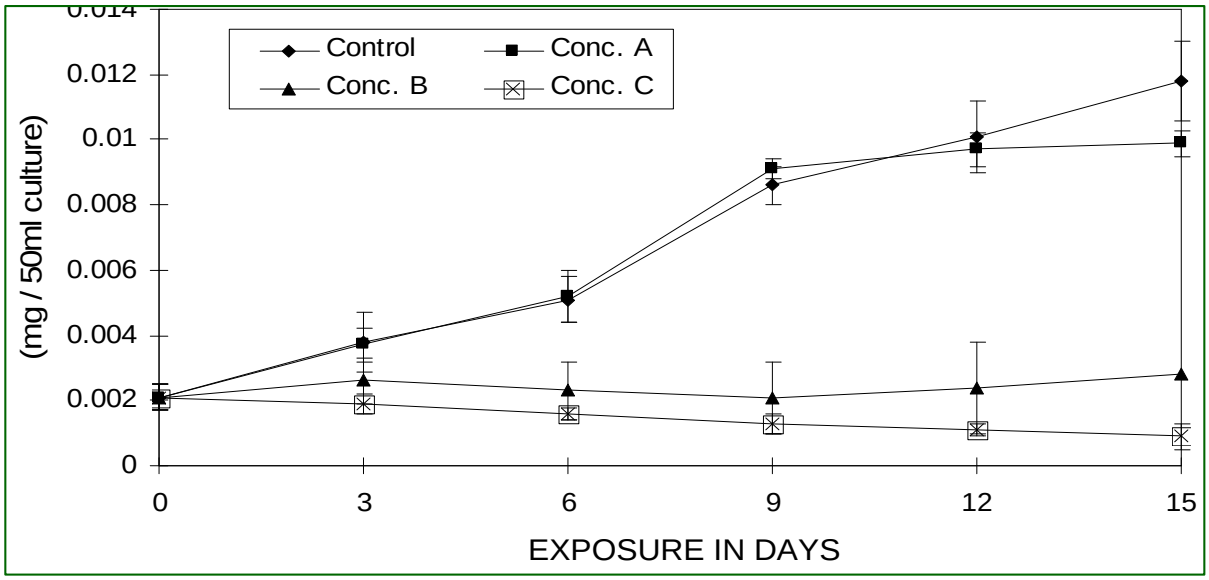


Fig. 8: Percent change in carotenoid content in Sevin exposed *Anabaena cylindrica,* in comparison to respective control values, at different days of exposure.

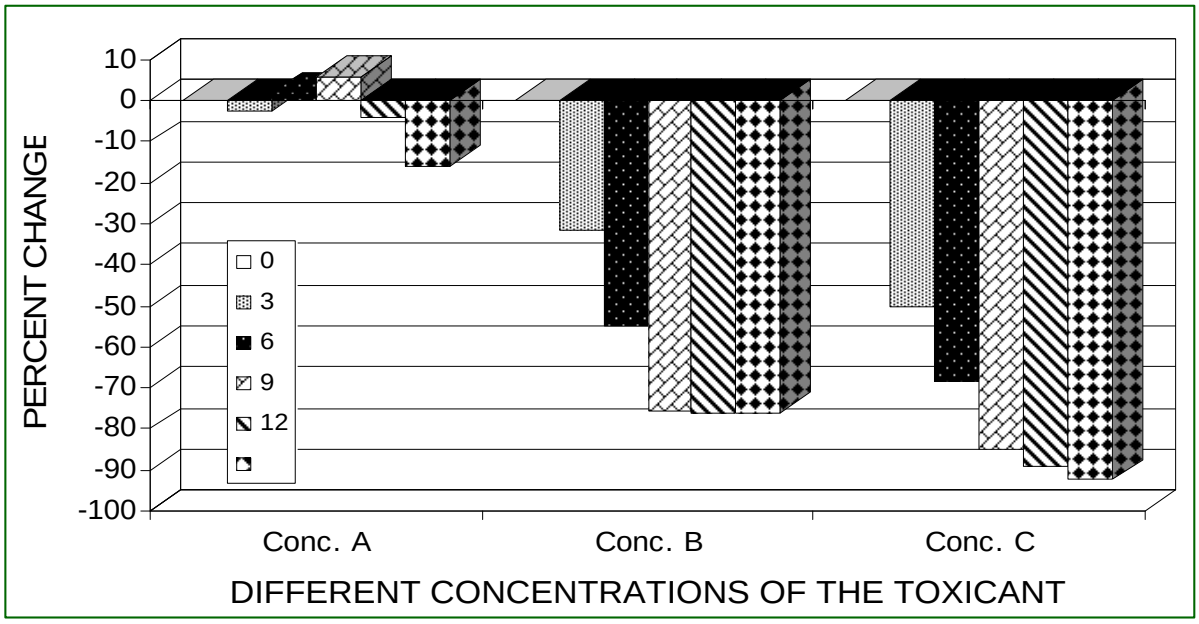
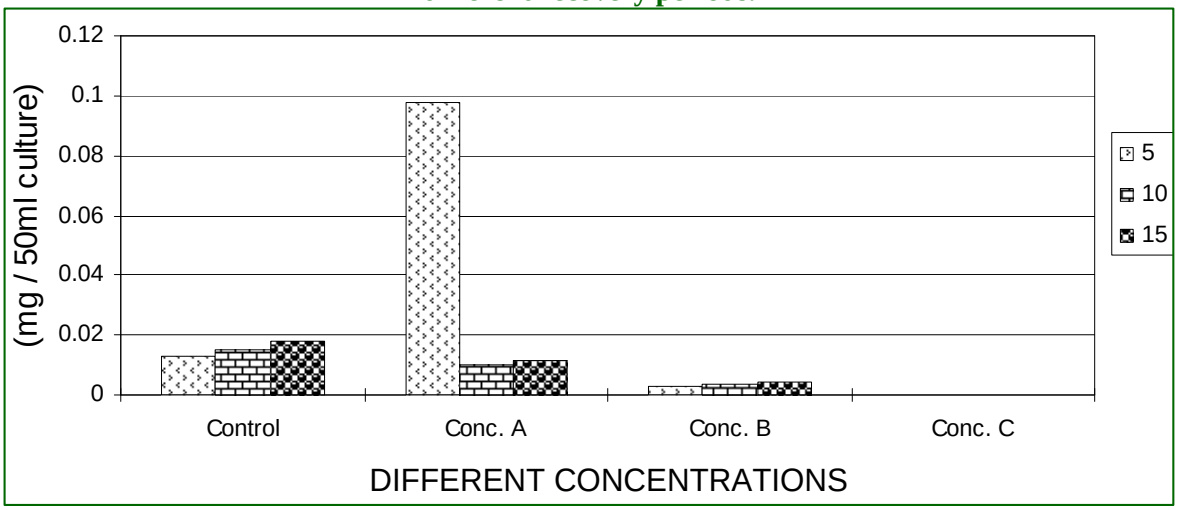


Fig. 9: Changes in total carotenoid content in control and Sevin exposed *Anabaena cylindrica* at different recovery periods.



The correlation coefficient analysis between days of exposure verses total Chlorophyll indicated the existence of significant positive correlation in control (r = 0.993, $p \leq 0.001$), Conc. A (r = 0.971, $p \leq 0.01$) and Conc. B (r = 0.991, $p \leq 0.001$) and a negative correlation (r = -0.915, $p \leq 0.05$) was marked in Conc. C (Table 2). In between percent change in chlorophyll content verses days of exposure were all negative significant ($p \leq 0.01$) in Con. A and B but in case conc. C, highly significant ($p \leq 0.001$) correlation was marked.

In between phaeophytin content and days of exposure indicated the existence of positive and highly significant ($p \leq 0.001$) correlation in control, conc. A and in conc. B significant correlation ($p \leq 0.01$) was marked. But in case of conc. C a non-significant negative correlation was marked in concentration C (r=-0.451, *p*=NS). The percent change in phaeophytin content showed the existence of negative significant correlation in all the three concentrations (A, B, C) studied In between carotenoid content of control and exposed alga verses days of exposure indicated the existence of a positive correlation and the values

were highly significant ($p \leq 0.001$), in control, conc. A and conc. B (Table 2). A negative and significant (r = - 0.935, $p \leq 0.01$) correlation was marked in conc. C. The percent change in carotenoid content versus days of exposure in conc. A, showed a non-significant negative correlation and in case of conc. B and C, showed a negative but significant ($p \leq 0.05$) correlation.

Table 2. Correlation co-efficient (r) between days of exposure and different parameters of study of *Anabaena cylindrica*, exposed to three different concentrations of the insecticide and control (NS = Not significant).

Concentration of the insecticide ml l^{-1}	Total chlorophyll content	Percent change in total chlorophyll	Carotenoid content	Percent change in carotenoid content	Total phaeophytin content	Percent change in phaeophytin content
Control (0.0) $p \leq$	0.993 0.001	-----	0.986 0.001	-----	0.997 0.001	-----
A (2.5ml l^{-1}) $p \leq$	0.971 0.01	- 0.939 0.01	0.985 0.001	- 0.516 N S	0.991 0.001	- 0.918 0.01
B (3.0ml l^{-1}) $p \leq$	0.991 0.001	- 0.957 0.01	0.991 0.001	- 0.923 0.05	0.916 0.01	-0.995 0.001
C (3.25ml l^{-}) $p \leq$	- 0.915 0.05	- 0.996 0.001	- 0.935 0.001	- 0.978 0.05	- 0.451 N S	- 0.984 0.01

Discussion

Pesticides which are used for preventing or destroying pest is having more negative impact on our ecological system when compared to its desired action. Pesticides are carried by wind and also by rain runoff water from the crop fields to other areas and contaminate them. This rain runoff water also contaminated the water bodies causing serious water pollution problems. Many of the chemicals used in pesticides are persistent soil contaminants, whose impact may endure for decades and adversely affect soil conservation (USEPA, 1989, 1996, 2000 and 2001). The use of pesticides decreased the general biodiversity in the soil. The assessment of toxic chemicals depends on two basic items of information. First, the environmental available concentrations of the chemical which occur as a result of discharge and distribution in the natural environment and secondly, the toxicological properties of the chemical at that concentration and its possible impact on the biota including humans. It is clearly evident that, Sevin an insecticide which is well known for its interference in biochemical metabolism becomes phytotoxic when used in higher concentrations. Thus, we can conclude that even though many advantages have been derived from the use of pesticides, more investigation is necessary to provide less toxic chemicals, non chemical approaches and more efficient methods of pesticide applications.

Many similarities exist in biological process among all forms of life, and investigative effects of this nature are not only important to plants, fish and wildlife, but to all life forms, including man. However, as long as the pesticides are used for pest control, there is always the possibility in future that unexpected and undesirable complication may arise even though minimum immediate hazards are occurring at present. If every person who uses pesticides will follow the simple recommendation "use only when absolutely necessary, choose the most suitable chemical, use the minimum quantity and apply through the best method available", environmental problems with regard to the application of pesticides will significantly reduce.

Safer application of these insecticides in the crop fields, at proper doses /concentrations also will not harm the non-target systems like nitrogen fixing, heterocystous blue green algae. These tiny particles

perform a fantastic job of atmospheric nitrogen fixation, increasing the fertility of paddy fields, maintaining the balance, used as biofertilizer. Due to careless handling of chemicals, heavy fumigation / mass spray, using broad spectrum killer chemicals will significantly harm the man made agricultural ecosystem.

Conclusion

Drastic depletion in chlorophyll content in exposed cultures only indicated either the destruction of the chlorophyll molecule or non-synthesis of chlorophyll in exposed algal cultures. At lower concentration of the toxicant (Conc. A), an initial higher value of chlorophyll content up to 9th day of exposure followed by depletion in the parameter at higher exposure period was noted. The phaeophytin content declined significantly and a maximum of 87.91% decrease was recorded on 15th day of exposure. Further depletion up to 97.35% was recorded during recovery studies. Significant depletion in carotenoid content in exposed cultures to the tune of 92.4% on 15th day of exposure and further depletion by 98.9% on 15th day of recovery and its non-recovery during recovery period indicate total destruction of the pigment. The pigment ratio value in Conc. A was much higher than the control organism.

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