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Activity of an Enzyme ATPase in an Alga *Anabaena cylindrica*, Lemm. with a Pesticide Sevin Under Laboratory Condition

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ABSTRACT

Pesticides or agro-chemicals play a pivot in controlling pests on agricultural and horticultural crops. SEVIN 50% W.D.P. based on carbaryl. 1. Naphthylmethyl carbamate, is a broad spectrum pesticide for control of pests on fruits, vegetables, forage, cotton and other crops, as well as poultry and pets. In order to know the extent of toxicity five different concentrations of the toxicant (Sevin) are taken – LC_0 (2.13 ml. l^{-1})) , LC_{10} (2.54 ml. l^{-1})), LC_{50} (3.01 ml. l^{-1}) , LC_{90} (3.25ml. l^{-1}) , LC_{100} (3.35 ml. l^{-1}) . Uni - algal, axenic culture of *Anabaena cylindrica*, Lemm. was inoculated and the survival percentage was determined. The (LC_{10} ,LC_{50} , LC_{90}) three lethal concentrations were chosen to study the differential effects of different concentrations of the pesticide (Sevin) on the blue-green alga, *Anabaena cylindrica*, Lemm. The changes in the ATPase activity (µg ip liberated hr $^{-1}$ 50 ml culture) of the alga *Anabaena cylindrica*, exposed to different concentrations of the pesticide, Sevin, at different days of exposure and recovery. The enzyme activity values were far less than the control value and even less than the inoculation day value, except the 3rd day of exposure. No significant recovery was marked, indicating total destruction of the enzyme system in the exposed alga. The percent change in ATPase activity showed non-significant negative correlation with the exposure period in conc. A and B. However, in conc. C, a negative significant ($r= -0.974$; $p \leq 0.01$) correlation was marked. The ATPase activity increased in Conc. A (2.5 ml l^{-1}) up to 12th day of exposure, when compared to the control value and then the enzyme activity decreased. With the increase in pesticide concentration and exposure period, the enzyme activity significantly declined, when compared to the control value. A maximum of 93.5% and 99.1% decrease was recorded on 15th day of exposure and 15th day of recovery in conc. C.

Introduction

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; and also rodenticides, nematicides, molluscicides and acaricides fall into their purview. A closely related group of insecticides are the carbamate esters first discovered by the Geigy Company in Switzerland in 1947, although the most generally effective member of the group carbonyl or Sevin (N-methyl a-naphthylcarbamate) was not introduced until nearly a decade later.

When pesticides are introduced into the environment many physico-chemical and biological forces begin to interact with them. As pesticides have high biological affinity, they play a vital role in understanding the behavior of pesticides. Assuming that pesticides are applied to soil or aquatic systems, one can immediately foresee two independent effects:

1. Adsorption-binding to soil or aquatic sediment particles and
2. Interaction with biological material.

The blue-greens die or affected because of the pesticides applied in recommended doses and higher doses due to carelessness of the illiterate user. *Anabaena cylindrica*, Lemm., a blue-green alga is found abundantly in the crop fields contaminated with the pesticides. It is a prokaryote, good fixer of atmospheric nitrogen, and prefers slightly alkaline pH and water logged conditions, for its growth and multiplication (Fogg *et al.*, 1973).

Keeping in view: the entry of pesticides into the crop fields, through mass spray or fumigation / periodic spray / varieties of spray and their possible effect on the nitrogen fixing blue-green algae in the crop fields; this paper was designed to evaluate the eco-toxicological effects on enzyme ATPase of the pesticide, Sevin on the blue-green alga, *Anabaena cylindrica*, Lemm.

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, 1976).

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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, is a broad spectrum pesticide for control of pests on fruits, vegetables, forage, cotton and other crops, as well as poultry and pets. 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 concetrations 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 upto 15 days.

Estimation of ATPase activity

The 50 ml culture solution of the flask was first centrifuged and the residual algal material was taken and homogenised with 5 ml of 0.25 M sucrose in a micro-tissue homogeniser and the homogenate was taken as the enzyme extract. One ml of the homogenate was added to two reaction vessels each (control and experimental) containing 5 ml of Tris buffer (7.4 pH). 1 ml of 5 M $MgCl_2$ & 1 ml of 0.1 M ATP was added to the experimental vessel. Both the reaction vessels were incubated at 37^0C for 60 minutes. After one hour the reaction was terminated by adding 7 ml of 10% TCA to each vessel. Samples were then transferred for 15 minutes to a refrigerator at 5^0C to allow complete precipitation of the homogenate proteins. The precipitate was then sedimented in a low speed (Remi-T8) centrifuge at 4000 rpm for 5 minutes. After centrifugation, the supernatant was taken to measure total ATPase activity.

Activity of ATPase was determined in algal tissues by measuring the amount of inorganic phosphate produced when adenosine triphosphate was converted to adenosine diphosphate. Total ATPase activity was measured with Na^+, K^+, Mg^{++} in reaction mixture. Inorganic phosphate produced as a result of the cleavage of ATP to ADP was measured by the method described by Martineck (1970). Colour development proceeded at room temperature for 30 minutes. Then the optical density was measured at 600 nm by using a spectrophotometer (Systronics). The ATPase activity was expressed as mg ip (inorganic phosphate) liberated hr $^{-1}$ 50 ml culture solution.

Results

The three lethal concentrations 2.13 ml/L,2.54ml/L, 3.01 ml/L,3.25 ml/L ,3.35ml/L were chosen to study the differential effects of different concentrations of the pesticide (Sevin) on the blue-green alga, *Anabaena cylindrica*, Lemm. Fig. 1 .represent the changes in the ATPase activity (μg ip liberated hr $^{-1}$ 50 ml culture) of the alga *Anabaena cylindrica*, exposed to different concentrations of the pesticide, Sevin, at different days of exposure and recovery.

In the control set, the ATPase activity increased from 18.6 ± 2.4 to 98.2 ± 2.2 μg ip liberated hr $^{-1}$ 50 ml culture medium within 15 days of exposure and further increased up to 142.4 ± 5.8 μg ip liberated hr $^{-1}$ 50 ml culture.

Whereas, in conc. A (LC_{10}), the value though showed regular increase from 18.6 ± 2.4 to 94.6 ± 7.5 μg ip liberated hr $^{-1}$ 50 ml culture within 15 days of exposure but 3.66% decrease in the enzyme activity was recorded on 15th day of exposure. The enzyme activities up to 12days of exposure were more than the control value at all exposure periods. In conc. B, the value depleted to 27.9 ± 1.7 μg ip liberated hr $^{-1}$ 50 ml culture on 15th day of exposure. In conc. C, the enzyme activity further depleted from 18.6 ± 2.4 to 6.4 ± 1.1 μg ip liberated hr $^{-1}$ 50ml culture, on 15th day of exposure. Interestingly, all the enzyme activity values were far less than the control value and even less than the inoculation day value (Fig.28), except the 3rd day of exposure. In the control set, the total ATPase activity increased with the increase in exposure period. At LC_{10} (2.54 ml l $^{-1}$), the ATPase activity showed decrease over the control value on 15th day of exposure. At LC_{50} (3.01ml l $^{-1}$), the activity further depleted when compared to control and conc. A., with the increase in exposure period, the enzyme activity declined significantly (Fig.28). At LC_{90} (3.25 ml l $^{-1}$), a drastic decline in enzyme activity was marked with the increase in exposure period. After 3rd day of exposure, the enzyme activity recorded, was less than the values on inoculation day ('0' day of exposure). Significant non recovery in enzyme activity was marked at A (LC_{10}). 'A' showed a regular increase in enzyme activity, whereas no effect in 'C' was marked (Fig.2) rather the values further depleted.

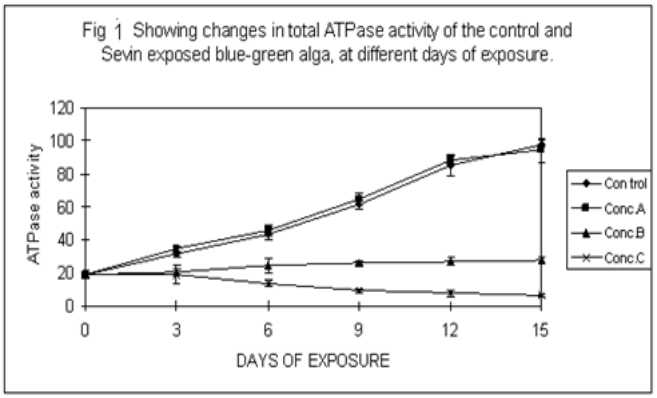
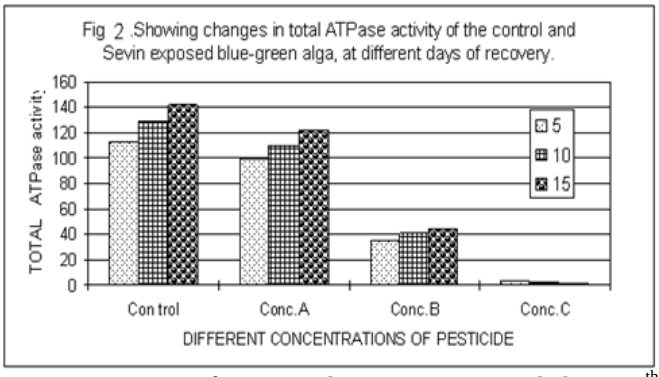
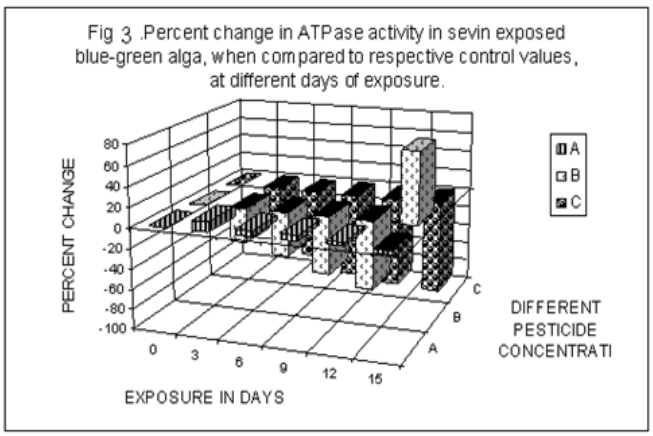


Fig.2 represented the percent change in the ATPase activity in exposed alga over the control value, at different exposure and recovery periods. The percent increase decreased from 9.87% to 3.51% on 12th day and a decrease up to 3.66% on 15th day of exposure leading to a steady decline in recovery period at 2.5 ml l $^{-1}$ Sevin concentration. In case of 'B' (3.0 ml l^{-1} of Sevin) decrease in the percent change was steady and drastic decrease in the enzyme activity was marked.



A maximum of 71.56% decrease was recorded on 15th day of exposure. The recovery, though not significant, showed a slight increment over previous values, indicating a

partial recovery in the activity of the exposed alga at 15 days of recovery (Fig.2). Whereas, in case of concentration 'C' (3.25 ml l^{-1}) a drastic depletion in enzyme activity was marked with the increase in exposure period when compared to the control value. No significant recovery was marked, indicating total destruction of the enzyme system in the exposed alga. Maximum 71.58% and 93.48% decrease in the ATPase activity was marked in exposed alga at conc. B & C respectively (Fig.3).



Maximum decrease was recorded on 15th day of recovery, when the exposed alga was transferred to toxicant free medium. Maximum of 69.17% and 99.08% was noted on 15th day of recovery in conc. B and conc. C, respectively. The correlation coefficient analysis between days of exposure and ATPase activity indicated the existence of positive significant correlation in control ($r = 0.995$, $p \leq 0.001$), conc. A ($r = 0.983$, $p \leq 0.001$) and conc. B ($r = 0.994$, $p \leq 0.001$) and a negative significant ($r = -0.956$, $p \leq 0.01$) correlation in conc. C. The percent change in ATPase activity showed non-significant negative correlation with the exposure period in conc. A and B. However, in conc. C, a negative significant ($r = -0.974$; $p \leq 0.01$) correlation was marked (Table-1). The ANOVA test indicated the existence of significant difference between rows and columns. The ANOVA test indicated the existence of significant difference between rows and non-significant difference between columns.

Table 1. Correlation co-efficient (r) between days of exposure and different parameters of study of the algae, exposed to three different concentrations of the pesticide and control. (NS = Not significant).

Concentration of the insecticide ml l^{-1}	Total ATPase activity.	Percent change in total ATPase activity
Control (0.0) P ≤	0.995 0.001	-----
A (2.54ml l^{-1}) P ≤	0.983 0.001	- 0.385 N S
B (3.01ml l^{-1}) P ≤	0.994 0.001	- 0.492 N S
C (3.25ml l^{-1}) P ≤	- 0.956 0.01	- 0.974 0.01

Discussion

It was found that the inhibition in the ATPase activity was dependent on the concentration of the stress and the period of exposure. There was a significant variation at different concentrations of the stress and at different exposure periods. Reduction of this symbiotic chemical signaling results in reduced nitrogen fixation and thus reduces crop yields (Deepa *et al*, 2008).

Inhibition in the activity might be affecting the membrane permeability and change in volume (De Filippis & Pallaghy, 1976 c). In exposed algal cells, drastic decline in ATPase activity was marked. The exposed alga showed inhibition and the same cannot be correlated with differential tolerance capacity of the alga. Mercuric ions and their organic derivatives served as probes in investigations of membrane transport, when they were used to block carrier sites (White & Rothstein, 1973) and thiol groups in membrane proteins practically. The fact that mercurials inhibit Na^+, K^+-ATPase has been established through work concerning the effects of mercurial diuretics on the enzyme (Nachay, 1974). Henderson et al. (1979) studied the effects of mercurials on the partial reactions of Na^+, K^+-ATPase and reported that ethylmercury and methylmercury were shown to selectively inhibit Na^+, K^+-ATPase while not affecting K^+-ATPase, Na^+ -ATPase, ADP - ATP exchange reaction and phospho-enzyme formation. It has been reported that a significant depression of Na^+, K^+-ATPase is associated with excessive absorption of mercury (Jackim, 1974). Significant depression in ATPase activity in exposed cultures was marked here, when compared to control cultures.

Pesticides can cause permanent damage is well evident from this result. Fox *et al* (1975) reported a different type of effect with organic mercury. Oxygen uptake and $^{14}CO_2$ production were inhibited in a progressive fashion with increasing mercury concentrations. Barron *et al.* (1948), Shieh and Barber (1973) and Matsumoto *et al*. (1971) reported the increase in activities at lower concentrations of the toxicant which is totally in agreement with this finding. The inhibition of enzyme activity, decrease in photosynthetic efficiency (Rath *et al.*, 1985), and the report that mercury attacked cytochrome systems and electron transport systems and incorporated with enzyme making then non-functional, suggested the idea that chemicals at higher concentrations becomes toxic and lethal. This principle seems to be valid, when we consider the present findings.

Conclusion

Sevin, the pesticide showed significant difference in action at different concentrations on a blue-green alga, is well evident from the table and figures described in the result. At higher concentration of the pesticide and higher exposure period, drastic effects on the blue-green alga were observed. At the highest concentration (Conc. C) of the pesticide, the alga showed typical toxic symptoms, beyond which survival of the alga becomes extremely difficult and at times impossible under laboratory control conditions. The analysis of variance ratio tests and correlation coefficient analysis carried out for the present parameter studied indicate clearly that the pesticide, Sevin is extremely toxic to blue-green alga. The ATPase activity increased in Conc. A (2.5 ml l^{-1}) up to 12^{th} day of exposure, when compared to the control value and then the enzyme activity decreased. With the increase in pesticide concentration and exposure period, the enzyme activity significantly declined, when compared to the control value. A maximum of 93.5% and 99.1% decrease was recorded on 15^{th} day of exposure and 15^{th} day of recovery in conc. C.

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