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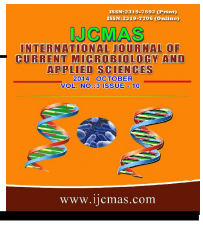

Original Research Article

Studies of Biochemical Stress in Blue Green Alga (*Anabena cylindrica*, L.) exposed with a Insecticide, Endosulphan, EC- 35

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A B S T R A C T

Anabaena cylindrical ,L a blue green alga commonly found in rice fields was experimentally exposed with a board spectrum insecticide,Endosulphan,EC50 in laboratory conditions for varied days. After exposure of the test alga , the biochemical parameters like Free Amino Acids(FAA),Protein, DNA and RNA were estimated to study the toxic effect and the subsequent recovery from insecticide stress. The results obtained indicated decrease in all studied parameters with increase in exposure period and concentration. The highest concentration (3.25ml/L) and highest period (15Days) severely affected the biochemical parameters and decreased the FAA, Protein, DNA and RNA content in alga with exception to 2.5 ml/L insecticide concentration, where it stimulated the studied parameters. However, when the algae were allowed to grow in insecticide free environment for 15 days, they could able to recover fully from the toxic stress.

Keywords

Endosulphan, alga, biochemistry, stress

Introduction

The use of toxic chemicals as killer chemicals together with fertilizers and improved hybrid crop varieties has a greater contribution to higher yield in agriculture. With the fast growing population of the world, a drastic increase in food production is the immediate need, where the use of pesticides to check the destruction of food seems to play an important role. However, the extensive use of pesticides created a wide range dilemma pertaining to its mode of action and behavior pattern on all other biosystems, present in the exposed /contaminated ecosystem. In modern methods of agricultural practices, though many organophosphorus, organochlorine, carbamate and other chemical pesticides have been introduced to disinfect the various seeds, the organomercurial fungicides are still to-day considered to be the potent seed dresser as these compounds are able to eradicate inoculums from the seed. The chemicals have both the fungicidal and bactericidal properties. Many varieties of modern synthetic killer chemicals (pesticides) are now available in the Indian

market, under different brand names. In a crop field, atleast 2 to 3 varieties of pesticides are sprayed/fumigated in a season depending on the type of crop under cultivation. In a year at least we go for 2-3 crops in the same agricultural field. Hence, theoretically and also practically, 6-8 types of pesticides are sprayed in a year. These pesticides after spray, gets deposited on the agricultural field. In the rainy season or by rain, the pesticides sprayed over the plant are drained into the crop filed.

Anderson (1978) reported that the wide use of certain pesticides has more serious and permanent drastic effect on microorganisms. It is well established fact that nitrogen-fixing organisms, particularly blue-green algae, are known to play a key role in increasing soil fertility, especially in paddy fileds under water logged condition (De, 1939, Singh, 1961; Stewart, 1977; Pattnaik, 1966). Thus, the pesticides which enter into the paddy field might be affecting the growth and nitrogen fixing capacity of the blue-green algal systems. Wright (1978) reported that Anabaena cylindrica was more sensitive to higher doses were stimulative to algal growth. Clark & Wright (1970) reported the pigment extract of Tolypothrix tonuis and found a depleted level of phycoerthrin and chlorophyll-a, with the relative increase in phycocyanin in the presence of chloro-prophan.

Keeping in view of 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 project was designed to evaluate the effects on biochemical parameters with exposure of the pesticide, Endosulfan on the blue-green alga, Anabaena cylindrica, Lemm.

Materials and Methods

Test organism

Anabaena cylindrical, Lemm is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae.

Test insecticide and selection of concentration

35% EC Endosulfan (Broad Spectrum Insecticide Acaricide) ENDOCEL 35 E.C. is dark brown liquid consisting of Endosulfan technical 35 w.w solvent, emulsifier, and stabilizers: 65 w.w. It is used as broad spectrum insecticide acaricide to control aphide, Jassids, Thrips, Beetles weevils, foliar feeding larvae, mites borers, cutworms bugs white flies, scales, Termites and slugs on deciduous citrus and small fruits vegetables, field crops, oil crops, fiber crops, tobacco, tea, forests and ornamentals.

Selection of Concentration and duration

Three concentration of Endosulphan was selected were 2.5,3.0 and 3.25 ml/L and days of exposure were 0.3,6,9,12 and 15 days. After the exposure the alga were allowed to recover in insecticide free condition up to 15 days again.

Estimation of Biochemical parameters

The Biochemical parameters like Protein (Lowry et al., 1951), Amino Acids (More and Stein, 1948), DNA and RNA (Schmeider, 1957) of the test organism (Anabaena cylindrica) were estimated following the standard procedures.

Results and Discussion

In the present investigation, Table 1, is very clear, where no dichotomy, in biochemical

behavior was marked, induced by the pesticide. At all the three selected concentrations of the insecticide. Endosulfan, decline in biochemical parameters was marked. At concentration A, ((2.5 ml/L)), the decline in Protein, DNA and RNA of the alga was not significant. However, with the increase in exposure period, the exposed alga showed a positive trend with the increase in exposure period, when compared to the control value, a similar positive and significant trend. At higher concentration of Endosulfan (3.25 ml/L) significant and negative correlation was marked. This indicated that the concentration of the toxicant plays a critical role in pesticide toxicity.

The drastic decline in DNA, RNA and protein content was marked in Endosulfan exposed cultures indicate the inhibition DNA synthesis and protein synthesis. The increase in FAA at lower concentration of the insecticide might be due to breakdown of proteins and at higher concentration further disintegration FAA might be the reason of free-amino acid decrease.

Endosulfan an insecticide which is well known for its interference in biochemical metabolism becomes phototoxic 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 exists 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.

Studies of pesticidal effects on algae have mostly been restricted to laboratory based bioassays employing a single species grown axenically. Though this approach does give some insight into pesticide effects on algae, it cannot solely be used to simulate natural conditions, as in nature many interacting environmental and physiological factors affect and determine the action of toxicants.

The peculiar behavior of the algal organisms under stress condition to avoid the stress, in an interesting feature in toxicological studies. Due to exudation, the medium might be changing or the exuding chemical might be reacting with Endosulfan forming a hard cyst, which must be providing an adhering surface for the pesticide. The cyst might be restricting the insecticide's entry into these cells, due to the formation of barrier before the membrane.

In the present investigation, drastic decline in DNA, RNA and protein content was marked in Endosulfan exposed cultures indicate the inhibition DNA synthesis and protein synthesis. The increase in FAA at lower concentration of the insecticide might be due to breakdown of proteins and at higher concentration further disintegration FAA might be the reason of free-amino acid decrease.

Toxicants effect on macromolecular content is often due to an indirect action on nucleic acid and protein synthesis, since a toxicant that interferes with energy yielding reaction is indirectly an inhibitor of synthesis of RNA, DNA and protein (Holbrook, 1980).

Table.1 Changes in faa, protein, DNA and RNA contents in endosulphan, ec- 35 exposed blue green alga (Anabena cylindrica,L.) at different exposure and recovery periods (Data are expressed in Mean + S.D of three replicates)

ND :- not detectable

Conc.of toxicants	**Parameter**	**EXPOSURE IN DAY**						**RECOVERY IN DAY**		
		0D	**3D**	**6D**	**9D**	**12D**	**15D**	**5D**	**10D**	**15D**
Control	FAA	2.24±0.14	2.65±0.18	2.95±0.23	3.31±0.17	3.98±0.31	4.54±0.54	5.16±0.26	6.32±0.31	7.38±0.29
	Protein	0.06±0.02	0.28±0.04	0.35±0.03	0.47±0.05	0.50±0.02	0.71±0.04	0.79±0.07	0.86±0.05	0.91±0.06
	DNA	0.56±0.08	0.79±0.04	0.91±0.06	1.12±0.02	1.28±0.09	1.39±0.11	1.56±0.13	1.74±0.07	1.93±0.08
	RNA	0.36±0.06	0.54±0.05	0.73±0.04	0.96±0.11	1.22±0.08	1.86±0.06	1.91±1.14	2.14±1.10	2.66±0.09
A 2.5ml/L	FAA	2.24±0.14	2.71±0.22	3.14±0.36	3.94±0.42	4.12±0.22	5.06±0.31	6.26±0.41	5.98±0.22	5.01±0.14
	Protein	0.06±0.02	0.24±0.01	0.31±0.04	0.35±0.03	0.37±0.05	0.41±0.06	0.46±0.05	0.52±0.04	0.61±0.09
	DNA	0.56±0.08	0.81±0.06	0.93±0.11	1.13±0.06	1.26±0.03	1.31±0.04	1.39±0.02	1.42±0.05	1.54±0.06
	RNA	0.36±0.06	0.41±0.04	0.63±0.03	0.88±0.06	0.91±0.04	0.99±0.07	1.06±0.11	1.18±0.14	1.31±0.06
B 3.0ml/L	FAA	2.24±0.14	2.54±0.18	2.91±0.26	3.14±0.31	2.98±0.51	2.18±0.35	2.32±0.41	2.51±0.16	2.86±0.27
	Protein	0.06±0.02	0.17±0.04	0.9±0.03	0.23±0.05	0.21±0.03	0.21±0.09	0.24±0.03	0.26±0.09	0.33±0.05
	DNA	0.56±0.08	0.59±0.03	0.61±0.02	0.61±0.04	0.63±0.02	0.59±0.05	0.61±0.09	0.60±0.11	0.62±0.14
	RNA	0.36±0.06	0.37±0.03	0.39±0.02	0.41±0.05	0.44±0.07	0.46±0.04	0.51±0.03	0.53±0.09	0.56±0.11
C 3.25ml/L	FAA	2.24±0.14	2.38±0.19	2.11±0.14	1.67±0.22	1.13±0.19	0.62±0.09	0.40±0.05	ND	ND
	Protein	0.06±0.02	0.09±0.01	0.03±0.01	0.02±0.006	0.01±0.002	0.01±0.009	0.01±0.004	0.005±0.0002	ND
	DNA	0.56±0.08	0.57±0.04	0.43±0.02	0.22±0.05	0.18±0.02	0.09±0.01	0.04±0.008	0.02±0.004	0.01±0.005
	RNA	0.36±0.06	0.37±0.04	0.35±0.02	0.22±0.04	0.14±0.05	0.09±0.02	0.03±0.01	0.01±0.004	0.01±0.006

A toxicant is probably acting in such a manner if the chemical inhibits the synthesis of all three macromolecules by comparable levels in dose-response experiments (Holbrook, 1980). A considerable dose-dependent reduction in the RNA, DNA and protein content observed in this study also confirms the action of toxicants on macromolecular synthesis. A reduction in RNA content (relative to the control level) with concomitant protein and DNA reductions indicated that the toxicant stressed larvae had reduced net rate of protein synthesis, probably mediated through decline in RNA (Buckley, 1980; Love, 1980 and Shaw, 1987).

Though the RNA and protein generally decreased concomitantly with increase in the Endosulfan concentrations, RNA decreased at a faster rate, as a result there occurred a disturbance in the protein/RNA ratios. Protein/ RNA ratios. Protein/RNA ratios have been found to decrease during the periods of rapid growth (in the species of crustaceae) and to increase in the offspring of methodone-dosed rat (Dagg and Littlepage, 1972; Peters, 1977 and Shaw, 1987). All most all the parameters studied showed a declining trend only, in exposed cultures, when compared to control cultures. The decline in parameters can be correlated with the pesticide toxicity only, as the only difference between the control culture and exposed culture was the addition of the pesticide in exposed cultures. Hence, it can be safely concluded that the damage caused in exposed system was only due to the toxicant.

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