Bull. Environ. Contam. Toxicol. (1992) 48:223–229

Environmental Contamination and Toxicology

Differential Effect of Dimethoate Toxicity to *Anabaena doliolum* with Change in Nutrient Status

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Scientific investigations on the effects of pollutants in the natural environment has focused attention on the individual biological species. Such investigations can only give suggestions as to the likely effects of the pollutants on the complex ecosystems existing in the natural environment. Recently there has been increasing interest in the effects of pollutants on the ecosystem, as a whole, which is of primary management concern. Therefore, utmost importance is being given on the effects of agrochemicals on the nontarget organisms both in the aquatic and terrestrial environments. Among these nontarget organisms certain cyanobacteria, due to their planktonic nature and photoautotrophic growth, are exposed directly to agrochemicals. They occupy an important position in the ecological niche due to their ability to fix atmospheric nitrogen, as well as the organic matter which they provide. Therefore, any adverse effect on them due to the application of toxic chemicals might affect the economy of the soil and water. Biocides are used extensively in modern agriculture and so evaluation of their effects on microorganisms is essential. The regulation of toxicity of these chemicals by different environmental variables and the tolerance/resistance level of cyanobacteria is well-known. However, the information regarding the influence of nutrient concentrations on toxicity of the pesticide dimethoate to Anabaena doliolum is lacking. The present study deals with the variation in toxic effect of dimethoate to the cyanobacterium A. doliolum by changing the concentrations of certain nutrients in the culture medium.

MATERIALS AND METHODS

The heterocystous, nitrogen fixing and filamentous cyanobacterium Anabaena doliolum Bharadwaja was a clonal isolate of this laboratory. The filaments were cultured

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in sterile nitrogen free (C-N) modified Chu No. 10^{+} medium (Safferman and Morris 1964), which contained SO_4^{2-} (10.28 mg/L) and PO_4^{3-} (5.43 mg/L), with A_6 micronutrients (Allen and Arnon 1955). It contained $CaCl_2$ replacing $Ca(NO_3)_2$ of the normal Chu No. 10^{+} medium in order to have equivalent amount of Ca^{2+}. Ferric citrate and citric acid were used as iron and chelating agents, respectively.

The stock cultures were grown in 250 mL borosilicate conical flasks containing 100 mL of the culture maintained in a culture room at a temperature of 27 ± 2°C with light/dark 12/12 hr photosynthetic photon flux density (PPFD) 70 µmol/m^2 sec photosynthetically active radiation (PAR: 400-700 nm) using white fluorescent tubes. Exponentially grown cultures were used as initial inoculum for the experiment. The experimental cultures were also grown in the same conditions.

An organophosphorus pesticide, dimethoate [O, O-dimethyl-S-(N-methyl-carbamoylethyl)-dithiophosphate] was used as the test chemical which was made available from Rallis India Ltd., Bombay. Its stock solution (1 g/L) was prepared in the sterilized medium for repeated use. The chemicals, $MgSO_4.7H_2O$, K_2HPO_4 and KNO_2 were used as the source of SO_4^{2-}, PO_4^{3-} and NO_2^{-}, respectively. Their stock solutions(1 g/L) were also prepared in the sterilized medium. Various concentrations of dimethoate, SO_4^{2-}, PO_4^{3-} and NO_2^{-} in mg/L were added to the cultures growing at log phase (8 hr after inoculation) under sterile condition. The cultures were handshaken twice daily. Different concentrations of SO_4^{2-}, PO_4^{3-} and NO_2^{-} were added to the culture tubes having sublethal concentration of the pesticide. The sublethal concentration [20 ± 1.32 mg/L (p=0.05)] at which 50 % of the population survived, has been referred as LC50 in the text. In these sets of experiment the tubes containing only sublethal dose of the pesticide were used as control (referred as LC50-control in the text) while the tubes without pesticide as control in case of study of the effect of the pesticide alone.

The survival test of the alga was done on C-N agar plates with varying concentrations (5-50 mg/L) of dimethoate. Samples of 0.1 mL of the culture growing at exponential phase of growth (12 filaments/cm^2 or 1.885 x 10^3 cells/cm^2) were spread evenly on the plates and incubated in the culture room for 10 d. The survival rates were calculated by counting the number of colonies under a binocular microscope considering the survivability in the control as 100 %.

Borosilicate culture tubes (18 x 150 mm) were used in

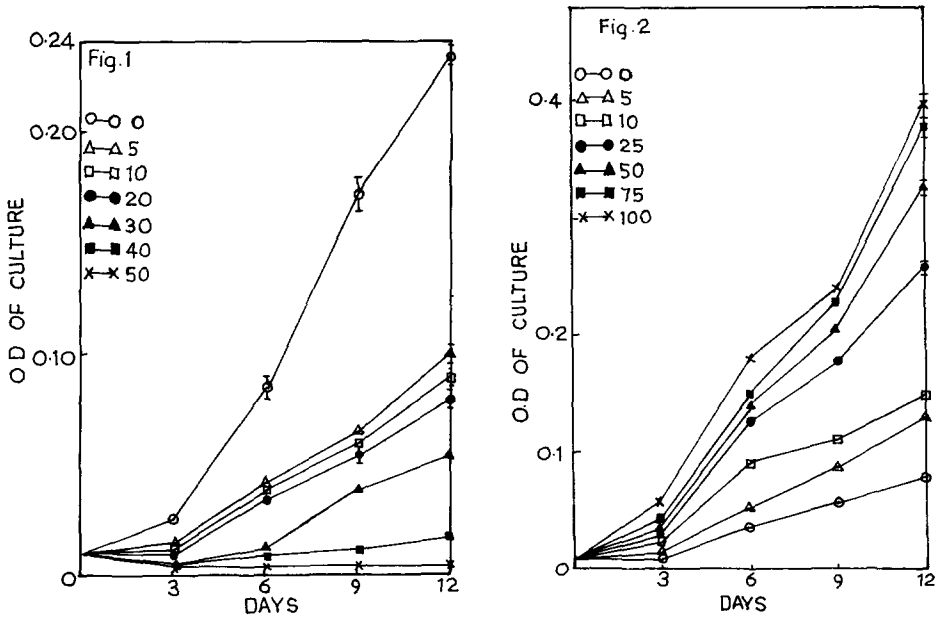


Figure 1. Effect of dimethoate concentrations (mg/L) on growth of Anabaena doliolum

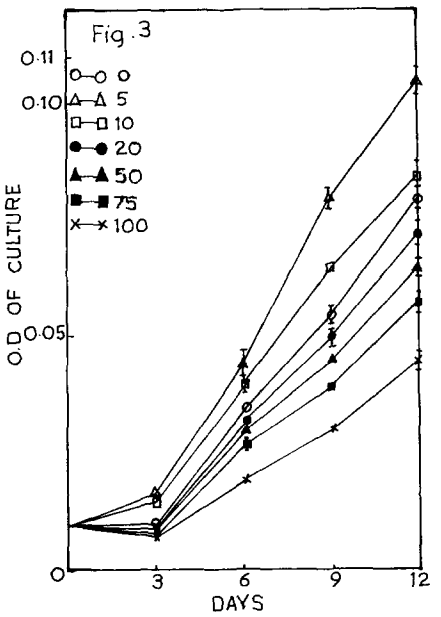
Figure 2. Effect of LC50 of dimethoate supplemented with No_2^- concentrations (mg/L) on growth of Anabaena doliolum.

the culture experiment. Optical densities (OD) of the homogenized cultures at 660 nm were measured every 3 d with the help of a Carl Zeiss SPEKOL spectrophotometer.

RESULTS AND DISCUSSION

The survival test revealed that dimethoate at 40 mg/L was algistatic and the other higher doses were algicidal. However, the survival rate was reduced by 26.5 % at 5 mg/L of the pesticide and there was gradual reduction in the survival from 5 mg/L to 50 mg/L . Between this range, 20 mg/L was found to be sublethal to the cyanobacterium since about 50 % (49.3 %) of the filaments survived at this concentration. At 40 mg/L of dimethoate, the survival rate was as low as 3 % of the control.

The time and dose dependent growth of A. doliolum in response to dimethoate was determined in liquid medium (Fig. 1). The cyanobacterium tolerated the same doses as those in the agar plates but compared to the survival rate the growth rate was highly reduced at 5 mg/L of the



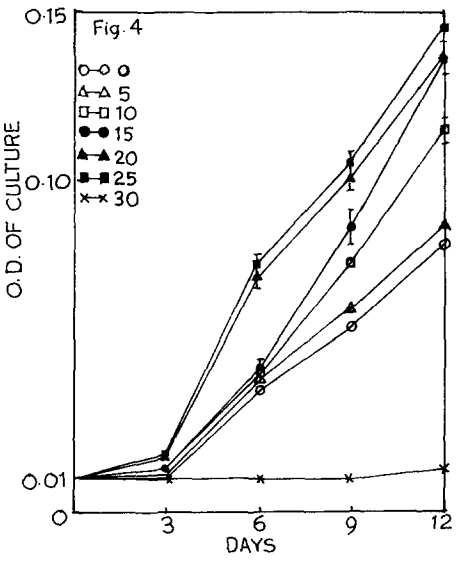


Figure 3. Effect of LC50 of dimethoate supplemented with PO_4^{3-} concentrations (mg/L) on growth of Anabaena doliolum

Figure 4. Effect of LC50 of dimethoate supplemented with SO_4^{2-} concentrations (mg/L) on growth of Anabaena doliolum

pesticide. After 12 d of incubation the growth rate at this concentration was 42.6 ± 1.49 % of the control. With further increase in dimethoate concentration, gradual reduction in the growth rate was observed and at LC50 the growth rate was 34 ± 1.49 % of the control after 12 d of incubation.

However, it is interesting to note that at LC50 of the pesticide, the growth rate of A. doliolum was accelerated with increase in the concentrations of the NO_2^- added to the cultures and toxicity effect of the LC50 to the cyanobacterium was reduced (Fig. 2). A concentration of even 5 mg/L of NO_2^- could accelerate the growth significantly at LC50. Direct relation of growth rate of the cyanobacterium and NO_2^- concentrations was time and dose dependent. After 9 d of incubation the rate of growth at 5 mg/L of NO_2^- was 154 ± 4.18 % of the LC50-control and after 12 d it was 162 ± 1.38 %. There was almost five fold increase in the growth rate, after 12 d of incubation, at 100 mg/L as compared to that in the LC50-control.

In case of PO_4^{3-}, only the lower doses like 5 mg/L and 10 mg/L (excluding its concentration in normal Chu No. 10 medium) were found to be useful in reducing the toxicity effect of dimethoate. The other higher doses reduced the growth rate, thus accelerating the toxic effect of the pesticide (Fig. 3).

Increase in SO_4^{2-} concentrations accelerated the growth of A. doliolum to a certain extent. Though very low dose like 5 mg/L (excluding its concentration in normal Chu No. 10 medium) could not show any significant rate of increase, other higher doses like 10-20 mg/L accelerated the growth rate to quite a considerable extent and thus reduced the effect of toxicity. However, the growth rate was highest at 25 mg/L of SO_4^{2-} where it was 181 ± 5.26 % of the LC50-control after 12 d of incubation and it did not increase beyond this concentration (Fig. 4).

Many aquatic organisms including both prokaryotes and eukaryotes respond differently to the agrochemicals with change in the population density (cells/mL) at initial level (Kar and Singh 1978; Mohapatra et al. 1990a). However, population density of the planktonic algae is directly dependent on growth rate which is controlled by environmental factors like light, pH and nutrient concentrations (Goldman 1986).

The present study reveals that dimethoate is highly toxic to A. doliolum even at tested low concentration (5mg/L). Concentrations like 40 mg/L and 50 mg/L are found as algistatic and algicidal, respectively, while 20 mg/L as sublethal.

The cyanobacterium shows more tolerance to the pesticide with addition of NO_2^- source to the medium in the control. At 5 mg/L, NO_2^- accelerates the growth of the organism to a significant extent at LC50 of the pesticide and the acceleration of growth rate from 154 ± 4.18 % to 162 ± 1.38 % of LC50-control after 9 and 12 d of incubation, respectively indicates that the effect of NO_2^- on the LC50 is time dependent. Since dimethoate reduces the nitrogen fixing ability of A. doliolum (Mohapatra et al. 1990b), nitrogen sources could be useful in protecting the cyanobacterium from toxic effect of the pesticide (Kashyap and Pandey 1982). It is also established that nitrogen sources accelerate the growth rate and population density of phytoplankton because nitrogen is used as potential nutrient for growth (Venkataraman 1979; Harrison et al. 1990).

Several workers have found that phosphorus determines the growth rate and chemical composition of micro-algae in general (Elgavish et al. 1980; Bisoyi and Singh 1988). In the present study, 5 mg/L and 10 mg/L of PO_4^{3-} accelerates the growth rate of A. doliolum at LC50 of

dimethoate. This increase in growth rate vis-a-vis tolerance limit of the cyanobacterium to the pesticide might be due to utilization of PO_4^{3-} as an auxiliary nutrient source. However, in this case the increase in growth rate is neither time nor dose dependent. Since dimethoate is an organophosphorus pesticide, higher PO_4^{3-} -P concentrations as well as high time exposure probably exerted additional stress on the test organism thus increasing the toxicity effect of the LC50.

On the other hand, a time dependent relationship with the tolerance limit of A. doliolum was observed in respect of SO_4^{2-} under the same conditions. Of the different SO_4^{2-} concentrations tested, 10 mg/L and 15 mg/L showed the most dependent reduction in the toxicity effect. At these two concentrations the growth rates, after 9 d of incubation, were 136 ± 4.54 % and 154 ± 7.27 % of the LC50-control, respectively, and after 12 d increased to 154 ± 4.32 % and 169 ± 3.64 %, respectively. This is probably the first report that additional SO_4^{2-} -S source can reduce the toxic effect of dimethoate to A. doliolum , a situation which may be helpful in the field. However, secondary effects of adding SO_4^{2-} to the environment should be considered.

It is known that nutrient status has direct impact on growth rate of the cyanobacaterium, which is proportional to population density. In its turn population density regulates the tolerance limit of this group of organisms to agrochemicals (Kar and Singh 1978; Mohapatra et al. 1990a). In this piece of research regulation of toxic effect of dimethoate to A. doliolum is due to change in population density of the cyanobacterium with change in certain nutrient concentrations. This strengthens the findings which suggest that regulation of toxicity effects of different pesticides and other toxic chemicals is influenced by nutrient sources (Somasundaram et al. 1987; Megharaj et al. 1989). It is quite possible to think that artificial favorable conditions can be manipulated by raising the nutrient level to a certain extent in the environment to overcome the toxic effect of the pesticide without hampering growth of the cyanobacterium. On the other hand, cyanobacteria are not considered the most desirable species in oligotrophic environments.

Acknowledgments. The authors thank Prof. P.C.Tripathy, Head of the Department of Botany, Utkal University for providing laboratory facilities and DSTE, Government of Orissa for the financial assistance.

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Received November 6,1990; accepted April 16,1991.