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Effects of Dimethoate on N_2-Fixing Cyanobacterium *Anabaena* PCC 7119

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Extensive literature is available on the interaction of organophosphorus insecticides with their target organisms as well as with vertebrates and soil bacteria and fungi. However, limited attention has been paid to their effects on cyanobacteria and eukaryotic algae, the primary producers in soil and water ecosystems. This work studies the effect of dimethoate --a widely used organophosphorus insecticide-- on growth, cellular composition and physiological processes of the cyanobacterium Anabaena PCC 7119 (grown under N2-fixing conditions).

MATERIALS AND METHODS

Axenic batch cultures of Anabaena PCC 7119 were grown in a nitrogen free medium previously described by Mateo et al. (1986) under 90 μE/m2.s continuous illumination at 26oC and bubbled with 2.5% CO2 enriched air. Dimethoate (O,O-dimethyl S-methyl carbamoyl methyl phosphorodithioate), technical grade, was obtained from the Spanish Customs Office (Madrid), dissolved in water and added to the culture medium to concentrations ranging from 20 to 300 mg/L depending on the experiment.

Growth was determined by optical density at 750 nm and expressed as mg dry weight/mL with the following regression line: mg/mL = O.D.750 x 0.3676 + 0.022812. Nitrogenase activity was determined by acetylene reduction according to Stewart et al. (1968) in a gas chromatograph Hewlett-Packard 5840A with a FID detector and Porapak N 80/100 column. Column temperature was 50oC and detector temperature was 150oC. The retention time for ethylene was 0.8 min and for acetylene was 1.3 min. At the beginning of every working session the equipment was calibrated with a standart of known concentrations of ethylene and acetylene.

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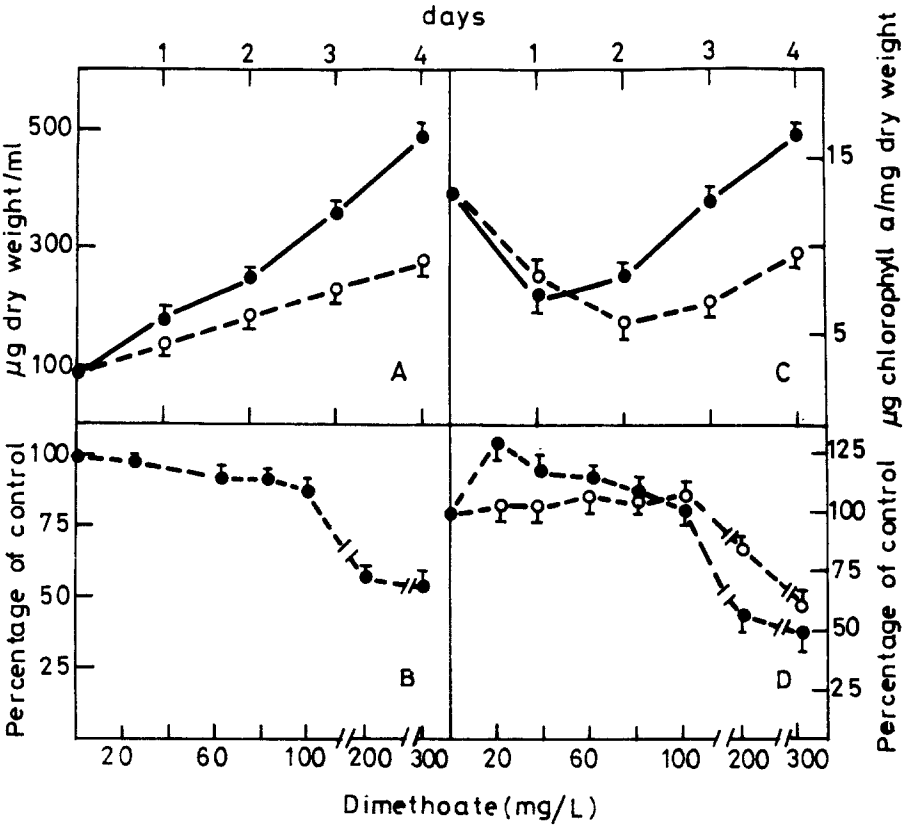

Figure 1. Effect of dimethoate on growth and cellular composition of Anabaena PCC 7119 . A- Effect of 300 µg/L on growth (●—●) control cultures; (○--○) Dimethoate-treated cultures. B- Dose dependence effect on growth after 72 hr of treatment. C- Effect of 300 µg/L on chlorophyll content. D- Dose dependence effect on photosynthetic pigments after 72 hr of treatment (●) chlorophyll a; (○) phycobiliproteins.

Photosynthetic O_2 evolution was measured with a Clark-type O_2 electrode of Hach Chemical Co. according to Blumwald & Tel-Or (1982). This electrode was calibrated with O_2 saturated water and having in mind the atmospheric pressure. Three mL aliquots of cell suspensions were placed and illuminated with a quantum flux density of 300 $\mu E/m^2.s$ in a temperature controlled cuvette. The same method was used for respiratory O_2 evolution but in darkness.

For chlorophyll determination, samples were extracted with 90 % methanol at 100°C for 3 min and estimated at 665 nm, according to Marker (1972). Phycobiliproteins were determinated at 620 nm in the supernatant of a suspension of cells treated with toluene during 4 hr in dark, according to Blumwald & Tel-Or (1982).

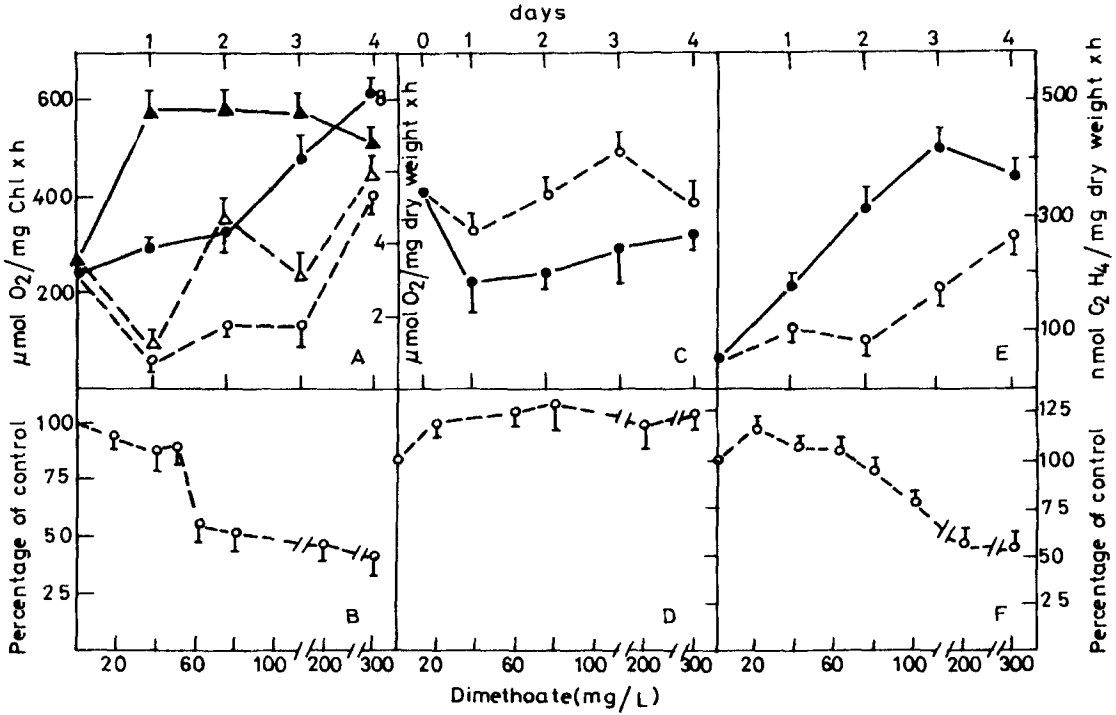

Figure 2. Effect of dimethoate on physiological processes of Anabaena PCC 7119: (●,▲) Control cultures; (○,△) dimethoate-treated cultures. A.- Effect of 300 mg/L on O_2 photoevolution in µmol O_2/mg Chl x hr (●,○) and µmol O_2/mg dry weight x hr (▲,△). B.- Dose dependence effect on O_2 photoevolution after 96 hr of culture. C.-Effect of 300 mg/L on respiratory O_2 consumption. D.- Dose dependence effect on respiratory O_2 consumption after 96 hr of culture. E.- Effect of 300 mg/L on nitrogenase activity. F.- Dose dependence effect on nitrogenase activity after 24 hr of culture.

Data presented are the means and standard deviations from at least four experiments with duplicate samples within each individual experiment.

RESULTS AND DISCUSSION

The addition of 300 mg/L of dimethoate to cultures of Anabaena PCC 7119 causes alterations in the cells. Growth is affected from the outset of the treatment (Figure 1-A) and this alteration is accompanied by a decrease of photosynthetic pigments content (Figure 1-C) that is noticeable after 24 hr of culture. A dose dependence of these effects can be observed using different concentrations of insecticide (Figures 1-B and 1-D). Concentrations of dimethoate as high as 100 mg/L cause very little inhibition of growth, but some increase of the major pigments, chlorophyll a and phycobiliproteins, is observed. Higher concentrations (200 and 300 mg/L) cause a pronounced decrease both in the growth of the cultures and in the pigments content.

The study of foremost physiological processes reveals that the addition of 300 mg/L of dimethoate alters the photosynthetic O_2 rate from the early stage of treatment. This effect is remarkable both when the measures are referred to dry weight of the cultures and when referred to the chlorophyll content (Figure 2-A). Thus, the fall in the rate of photosynthetic O_2 evolution has not a direct relation with the decrease on pigments content. On the other hand, the addition of 300 mg/L of dimethoate causes an increase in the respiratory activity (Figure 2-C) perhaps as a compensation mechanism. A dose dependence effect can be seen again on both activities when different concentrations of insecticide are tested (Figure 2-B and 2-D). Concentrations until 60 mg/L cause a slight inhibition on photosynthesis while higher concentrations greatly inhibit it. On the contrary, respiration slightly increases with all concentrations assayed.

In relation to the other important physiological activity, nitrogen fixation, the presence of 300 mg/L of insecticide causes a remarkable inhibition on nitrogenase activity from the beginning of the treatment (Figure 2-E) but the inhibitory effect of dimethoate on nitrogen fixation is not significant at concentrations below 100 mg/L (Figure 2-F).

To our knowledge there are not previous references about dimethoate and Cyanobacteria, but some works about the effect of other organophosphorus insecticides on Cyanobacteria show similar effects to this study. Trichlorfon causes the same or more acussed effects on

Anabaena PCC 7119 (Marco et al. 1990) and fenitrothion and chlorpyrifos cause some inhibitory effects on different Cyanobacteria (Lal et al. 1987). The toxicity of organophosphorus insecticides towards Cyanobacteria varies from that of compounds producing deleterous effects at doses as low as 1 mg/L to that of well tolerated chemicals that do not alter the growth even at 100 mg/L or more (Lal 1982).

Other authors report a decrease of photosynthetic pigments as a result of the insecticide presence, like malathion that alters chlorophyll synthesis in the cyanobacterium Oscillatoria (Torres and O'Flaherty 1976) or phorate and demeton that decrease photosynthetic pigments content on five Cyanobacteria (Kausik and Venkateraman 1983; Thomas and Shanmugasundaram 1986).

Other studies report effects of different insecticides on nitrogen fixation of cyanobacteria: fenitrothion and chlorpyrifos inhibit nitrogenase activity on Anabaena and Aulosira (Lal et al. 1987) but temephos stimulates it (Wurtsbaugh and Aperson 1977).

Several insecticides have been shown to alter photosynthesis of microalgae either estimated as oxygen photoevolution or as ^{14}C fixation (Lal 1982; Lal et al. 1987). The negative action of dimethoate on Chlorella and Elodea has been explained through a mutagenic action that causes a lesion in the photosynthetic apparatus (Aphaseva et al. 1985).

Interference with photosynthetic electron transport resulting from the binding of methyl parathion to the photosynthetic membranes has been suggested to be the primary mechanism of action of this insecticide on the alga Chlorella (Lal & Dhanaraj 1985). Photosynthesis seems also to be the first target of dimethoate on Anabaena because concentrations of this insecticide up to 100 mg/L reduce photosynthetic O_2 evolution but do not reduce significantly photosynthetic pigments nor nitrogen fixation. In the case of the lower concentrations (up to 40 mg/L) the effect on the photosynthetic process would be balanced by the increase of pigments, repiration and nitrogenase activity. However, concentrations of 100-200 μg/L have also a severe effect on nitrogenase activity, pigments and, consequently, on growth.

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