Bull. Environ. Contam. Toxicol. (1992) 48:894–900

Environmental Contamination and Toxicology

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

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There are few works on the effects of organophosphorus insecticides on photosynthetic microorganisms. Data available show effects on growth, photosynthesis, chlorophyll concentration and ATP levels (Lal 1982, Padhy 1985). Cyanobacteria are important photosynthetic microorganisms because they contribute to soil fertility by carbon-fixation and specially N_2-fixation. The number of studies about interactions between these microorganisms and organophosphorus insecticides is very limited.

Acephate is a widely used organophosphorus insecticide in Spanish fields for the control of different insect pests (de Liñan 1987). This work studies the effects of acephate on growth, cellular composition and physiology of Anabaena PCC 7119 grown under N_2-fixing conditions. The possible role of this cyanobacterium on the insecticide removal has been also investigated.

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 m^{-2} s^{-1} continuous illumination at 26°C and bubbled with 2.5% CO_2 enriched air. Acephate (O,S-dimethyl acethyl phosphoramidothioate), technical grade, was obtained from the Spanish Customs Office (Madrid) and added to the culture medium to concentrations ranging from 60 to 300 mg L^{-1} depending on the experiment.

Growth was determined by optical density at 750 nm and expressed as mg dry weight mL^{-1} with the following regression line: mg mL^{-1} = $O.D._{750}$ 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

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and Porapak N column. Photosynthetic O_2 evolution was measured with a Clark-type O_2 electrode of Hach Chemical Co. according to Blumwald and Tel-Or (1982). Three milliliter aliquots of cells suspension were placed and illuminated with a quantum flux density of 300 $\mu E\ m^{-2}\ s^{-1}$ in a temperature controlled cuvette. The same method was used for respiratory O_2 evolution but in darkness.

Proteins were determined by the method of Lowry et al. (1951) in extracts obtained by treating the samples with NaOH 1N at 80°C for 1 hr. Nucleic acids were extracted with 0.5 N perchloric acid at 70°C for 1 hr and estimated according to Ogur and Rosen (1950). The content of carbohydrates was determined by the method of Dubois et al. (1956) in extracts of toluene treated cells. For chlorophyll determination, samples were extracted with 90 % methanol at 100°C for 3 min and estimated according to Marker (1972). Phycobiliproteins were determined at 620 nm in the supernatant of a suspension of cells treated with toluene, according to Blumwald and Tel-Or (1982).

The time-course of acephate (initial concentration 300 mg L^{-1}) was evaluated in cell-free control flasks and in flasks inoculated with an initial algal concentration of 50 μg of dry weight mg L^{-1}. At the times indicated 10 mL-samples were withdrawn, the insecticide was recovered with dichloromethane and transferred to ethyl ether. This extract was determined by gas chromatographic analysis according to Carlstrom (1972) in a Hewlet-Packard 5840A Chromatograph with a 6% QF1 Chromosorb G (AW-DMCS) 70/80 mesh columm and a FID detector.

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

RESULTS AND DISCUSSION

The addition of acephate at concentrations up to 200 mg L^{-1} does not affect the growth of the cultures of Anabaena PCC 7119 and a rather little inhibition can only be noticed at the highest concentrations assayed (300 mg L^{-1}) (Figure 1A and B). The algal content in chlorophyll a is not modified by this insecticide but all the concentrations tested increase the other major photosynthetic pigments, phycobiliproteins (Figure 1C and D). Neither the main organic fractions, proteins, nucleic acids and carbohydrates, show significant alterations with the acephate concentrations assayed (Table 1).

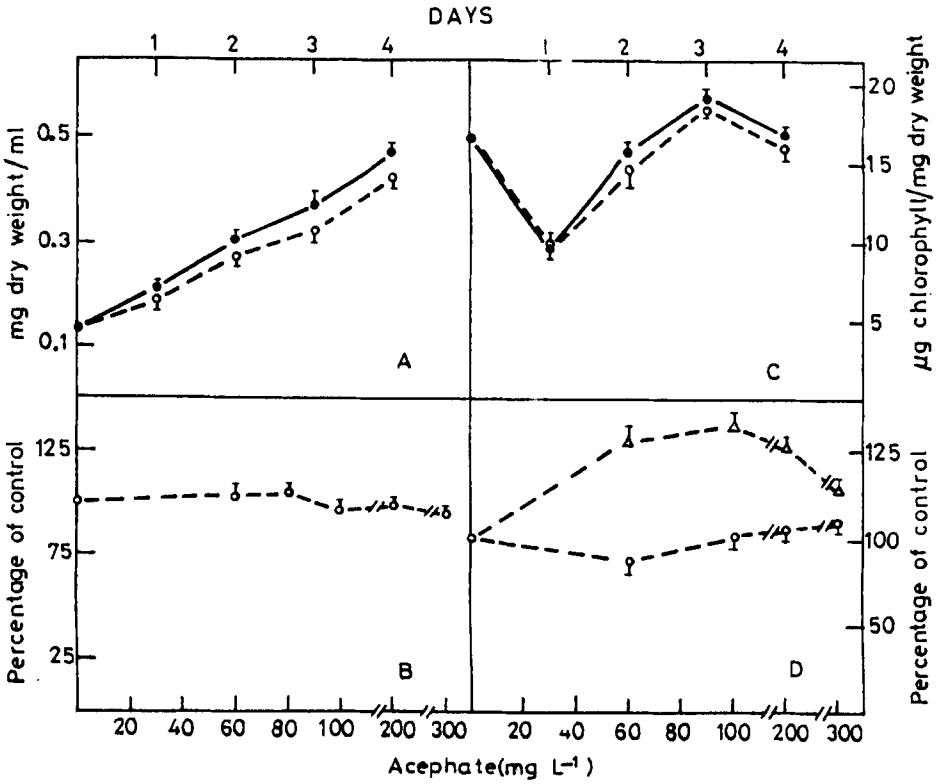

Figure 1. Effect of acephate on growth and photosynthetic pigments of Anabaena PCC 7119. A- Effect of 300 mg L^{-1} on growth. B- Effect of different concentrations on growth after 72 hr of treatment. C- Effect of 300 mg L^{-1} on chlorophyll a content. D- Effect of different concentrations on the cellular content of chlorophyll a (o), and phycobiliproteins (Δ) after 72 hr of treatment. (●) Control cultures, (O,Δ) acephate-treated cultures.

Table 1. Effect of different concentrations of acephate on main organic fractions of Anabaena PCC 7119 after 72 hr of culture. Data expresed as µg/mg dry weight.

	Control	50 mg L^{-1}	100 mg L^{-1}	200 mg L^{-1}	300 mg L^{-1}
Proteins	351.1±10.0	356.4±12.3	342.5±3.5	349.7± 6.4	352.8±22.0
Nucleic Acids	109.6±10.6	118.1± 5.2	110.9±12.7	114.0±18.3	108.4± 4.5
Carbohydrates	272.2±13.8	275.7±11.3	283.4±34.7	291.1± 2.7	300.3±17.4

However, some physiological alterations can be noticed in acephate-treated cultures of Anabaena. The addition of 300 mg L^{-1} of acephate provokes a decrease on O_2 photoevolution after 4 d of culture (Figure 2A) but concentrations lower than 200 mg L^{-1} do not produce significant effects on this activity (Figure 2B). A little increase in respiratory activity after 4 d of culture is observed with the different concentrations of insecticide assayed (Figure 2C and 2D) maybe as a compensation mechanism of photosynthesis. The treatment with 200 and 300 mg L^{-1} produces a clear decrease on nitrogenase activity at 24 hr of growth. The effect on this enzyme, that catalyses N_2-fixation, is transitory (Figure 2E), and low concentrations, till 100 mg L^{-1} do not affect nitrogenase (Figure 2F).

Microscopic examination revealed cell enlargement and a significant decrease in the number of cells of the cultures treated with 300 mg L^{-1} of acephate. The insecticide did not affect the frequency of heterocysts, the specialized cells where nitrogen fixation takes place in filamentous cyanobacteria.

The gas chromatographic measurements of the insecticide in cell-free control media and in cell-inoculated media show that there is not spontaneous degradation of acephate during 96 hr and that there are not appreciable differences between both media (Figure 3). These data indicate that Anabaena does not contribute to the removal of acephate from the medium.

Acephate does not cause a clear incidence on growth of Anabaena PCC 7119 even at concentrations as high as 300 mg L^{-1}. Just this concentration is the limit of a detrimental action. Accordingly, the effect of this insecticide is minimal and only slight alterations in the studied parameters can be observed. We have no reference of any previous work on the effect of this insecticide on cyanobacteria, but there are some reports on the effects of other insecticides on these microorganisms. Our results are in agreement with that of other insecticides assayed on different cyanobacteria showing no negative effects. Concentrations between 50 and 400 mg L^{-1} of Diazinon and Phorate caused no effects on Aphanothece (Kannaiyan 1980). Neither Diazinon produced alterations on Cylindrospermum and Aulosira (Singh 1973).

However, a great number of organosphosphorus insecticides showed a detrimental effect on cyanobacteria and other microalgae (Lal 1982, Padhy, 1985). In a previous work we have demonstrated that trichlorfon interferes with nitrogen fixation in Anabaena PCC 7119 and that this inhibition leads to a

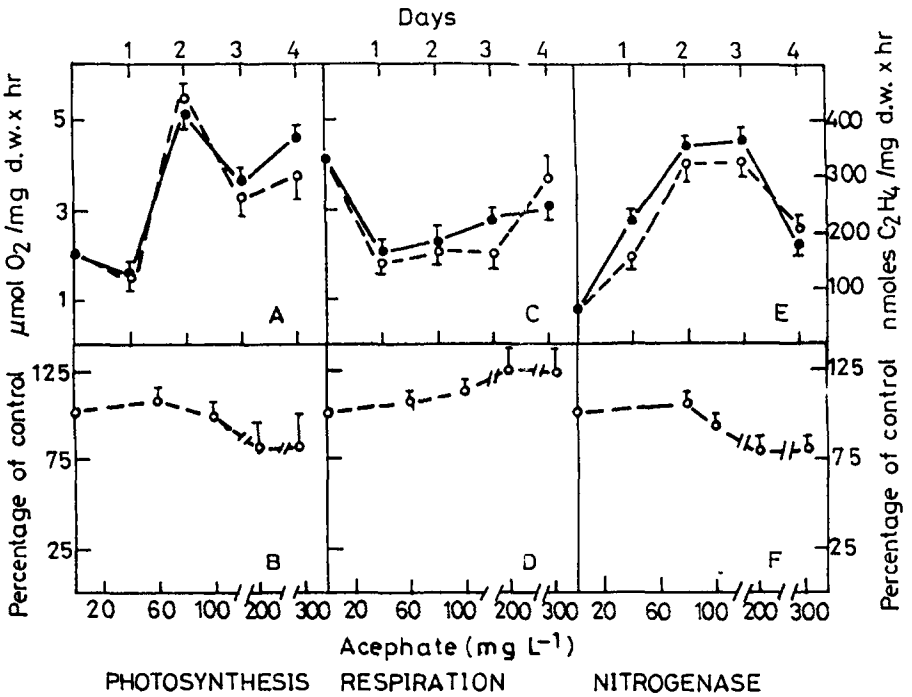

Figure 2. Effect of acephate on physiological processes of Anabaena PCC 7119. Effect of 300 mg L^{-1} on the time-course of A- O_2 photoevolution; C- Respiratory consumption; E- Nitrogenase activity. Effect of different concentrations after 96 hr of treatment on B- O_2 photoevolution; D- Respiratory consumption; F- Nitrogenase activity after 24 hr of treatment. (●) control cultures (○) acephate-treated cultures.

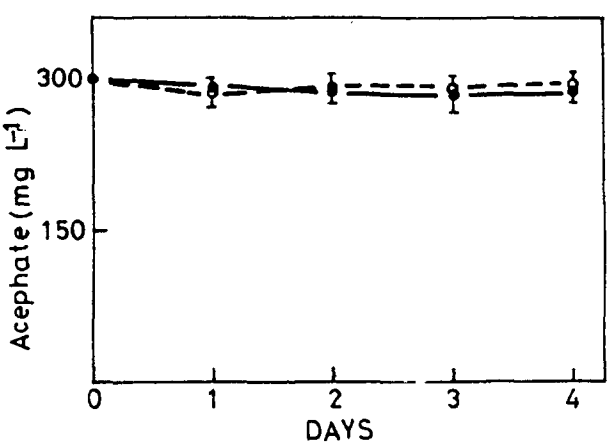

Figure 3. Time-course of acephate concentration in culture media. (●) cell-free media; (○) Anabaena inoculated media.

reduced amount of the nitrogenous photosynthetic pigments and a low photosynthetic rate, resulting in growth inhibition (Orús et al. 1990). Our present result shows that acephate also interferes with cell metabolism but only when assayed at high concentrations (200-300 mg L^{-1}) and, even in these cases, its toxicity is rather slight. The inhibition of nitrogen fixation is transitory and does not involve the reduction of photosynthetic pigments. The delayed and not remarkable effect on photosynthetic and respiratory rates can only be noticed after 96 hr of treatment. The fact that the effect of 300 mg L^{-1} on dry weight was negligible while the same concentration of insecticide provoked clear reduction of the number of cells accompanied by cell enlargement, indicated that acephate interferes with mechanism of cell division.

The absence of removal of acephate by Anabaena contrasts with the bacterium Pseudomonas, that is capable of metabolizing and utilizing this insecticide as sole phosphorus source (Rosenberg and Alexander 1979). As long as cyanobacteria are concerned, their ability to metabolize organophosphorus insecticides depends on the cyanobacterium and the chemical tested. Anacystis nidulans does not degrade parathion (Gregory et al. 1969), Aulosira fertilissima does not degrade malathion (Rao and Lal 1987) and Anabaena does not degrade trichlorfon (Orús and Marco unpublised data). On the contrary, Nostoc linckia, Synechococcus elongatus and Phormidium tenue are capable of metabolizing monocrotofos and quinalphos (Megharaj et al. 1987).

Our study suggests that no significant interactions can be expected between cyanobacteria and acephate under environmental conditions.

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Received July 10, 1991; accepted December 10, 1991.