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# Disappearance of Trichlorfon from Cultures with Different Cyanobacteria

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There are insecticides that are degraded mainly by chemical action and others that need biological action. The metabolism of organophosphorus insecticides by microorganisms is well-documented (Ware and Roan 1970; Greenhalgh et al. 1980; Lal 1982; Barik 1984). Among the microorganisms, bacteria and fungi are the major contributors to these processes whereas the studies of the role of photosynthetic microorganisms have been limited. Although microalgae have been involved in the metabolism of certain organophosphorus insecticides such as phorate, parathion, diphonate or diazinon (Lal 1982) information on the algal degradation of organophosphates is far from complete. Particularly, degradation involving cyanobacteria is little known.

This work studies the possible role of different groups of cyanobacteria in the removal of trichlorfon, a useful organophosphorus insecticide used in rice fields, where this type of microorganisms is abundant.

## MATERIALS AND METHODS

Axenic batch cultures of the unicellular Gloeothece PCC 6501, the filamentous Plectonema calothricoides and the filamentous with heterocysts Anabaena PCC 7119, Nostoc UAM 205 and Chlorogloeopsis PCC 6912 were grown in a C medium of Kratz and Myers (1955) under 60 $\mu E\ m^{-2}\ s^{-1}$ (25 $\mu E\ m^{-2}\ s^{-1}$ for cultures of Nostoc and 3 $\mu E\ m^{-2}\ s^{-1}$ for cultures of Gloeothece) continuous illumination at 26 °C and bubbled with 2.5 % $CO_2$-enriched air. Trichlorfon (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate), technical grade 97%, was obtained from the Spanish Customs Office and added to the culture medium to a final concentration of 300 mg $L^{-1}$.

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To evaluate the removal of this insecticide, 200 mL of culture medium in 0.5-L flasks were inoculated to give an initial algal concentration of 50 $\mu$g dry weight $mL^{-1}$ and treated with 300 mg $L^{-1}$ of trichlorfon. Control flasks without cells were prepared and kept under the same conditions and used to determine the spontaneous degradation of the insecticide. At 0 time and at regular intervals of 24 hr, 10-mL samples were removed from the control and from culture flasks and algae were pelleted by centrifugation or filtered. For polarographic determination samples were measured according to Giang and Caswell (1957) using an Amel 461 polarograph with an H-cell with a saturated calomel electrode in the anode compartment. The half-wave potential observed for trichlorfon was -0.68 volt. For gas chromatographic determination the insecticide of the media was recovered with dichloromethane and transferred to ethyl ether, being 89% the percentage of recovery. This extract was used for gas chromatographic analysis according to Anderson et al. (1966) using a Shimadzu GC-8A chromatograph equipped with a 16% GEXE 60 Chromosorb WAW 80/100 mesh column (6.35 mm I.D., 1.7 m) and a FID detector. Column temperature was 80 $^{o}$C and detector temperature was 270 $^{o}$C.The retention time for trichlorfon was 3.9 min.

Data in figures are the means and standard deviations from two independent experiments with duplicate cultures and duplicate samples within each individual experiment. The statistical significance of the data was estimated by means of a Student`s t test for $p<0.05$.

## RESULTS AND DISCUSSION

In order to know the role of cyanobacteria as biodegradating microorganisms of trichlorfon, the disappearance of the insecticide from the culture media with and without cells was measured. The results obtained by polarographic determination using three different cyanobacteria, Anabaena PCC 7119, Gloeothece PCC 6501 and Chlorogloeopsis PCC 6912, (Figure 1) did not show significant differences between cell-free media and media with cyanobacteria during the time of culture. This indicates that degradation of trichlorfon is due to physical and chemical conditions of the assays and biodegradation by these cyanobacteria does not exist.

However, in order to support with additional proofs this conclusion the same assays were carried out using gas chromatographic determination. Five cyanobacteria with different characteristics were used, the three

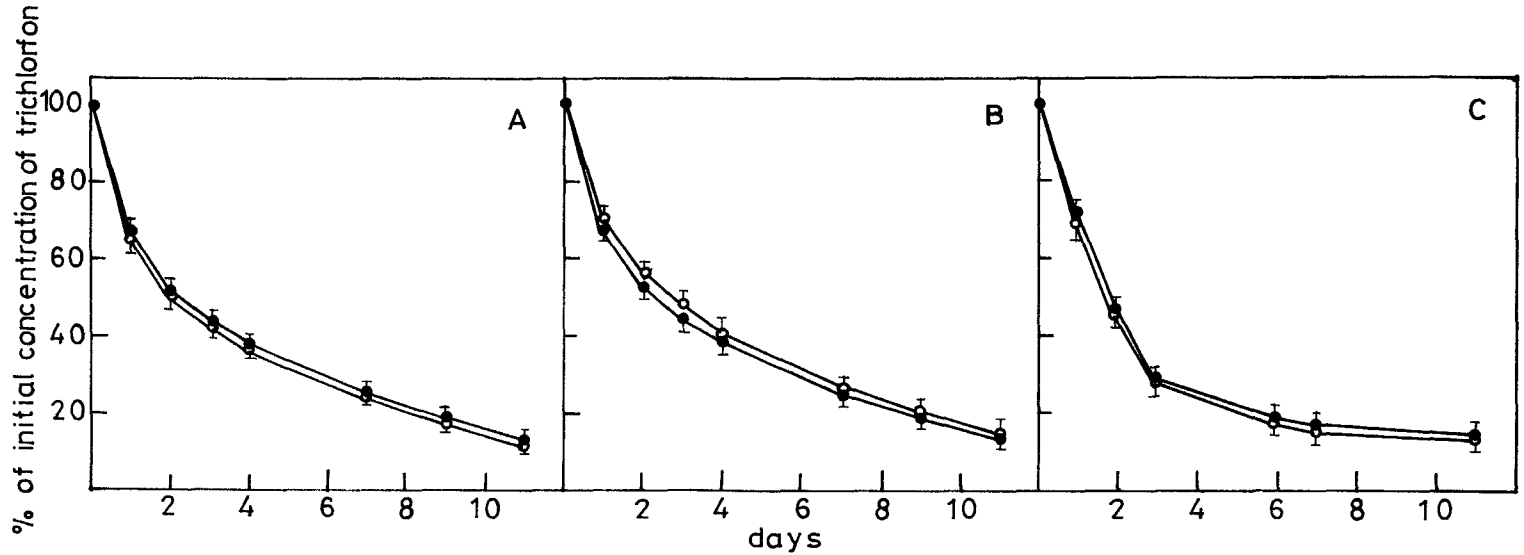


Figure 1. Disappearance of trichlorfon (initial dose 300 mg $L^{-1}$) from culture media with different cyanobacteria by polarographic determination. Data represented as percentage ±SD of the initial concentration of the insecticide in the media. A- Anabaena PCC 7119; B- Nostoc UAM 205; C- Chlorogloeopsis PCC 6912. Media without cells (●). Media with cells (o).

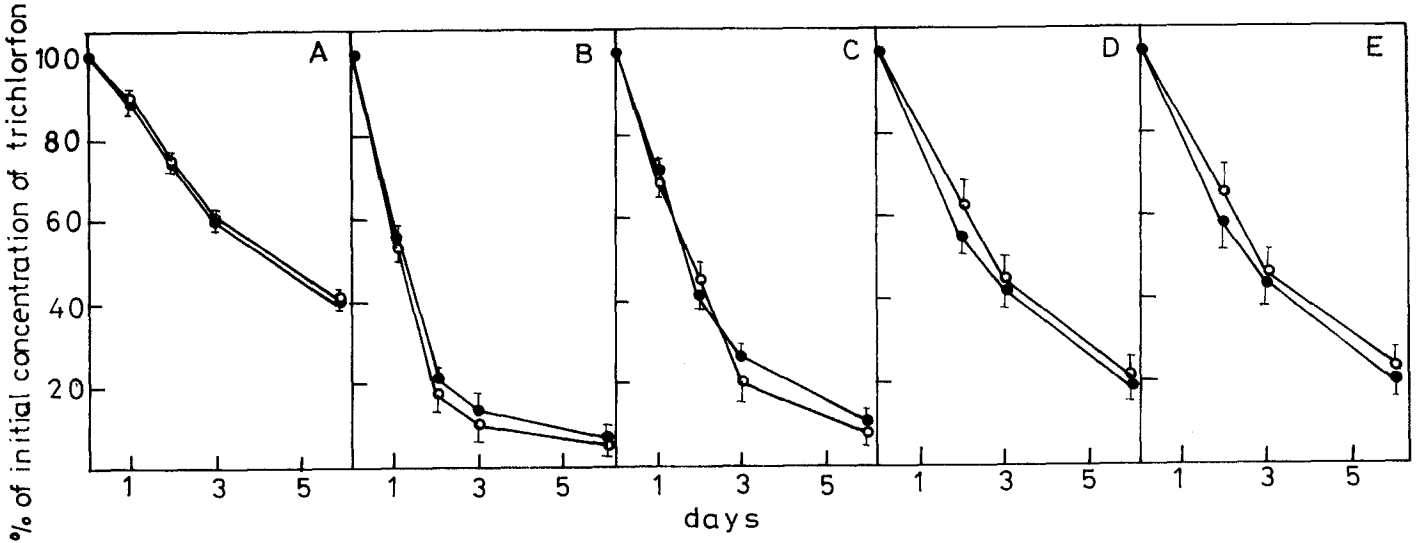


Figure 2. Disappearance of trichlorfon (initial dose 300 mg $L^{-1}$) from culture media with different cyanobacteria by gas chromatographic determination. Data represented as percentage ±SD of the initial concentration of the insecticide in the media. A- Anabaena PCC 7119; B- Nostoc UAM 205; C- Gloeothece PCC 6501; D- Chlorogloeopsis PCC 6912; E- Plectonema calothricoides. Cell-free control media (●). Cell-inoculated media (o).

preceeding ones plus Nostoc UAM 205 and Plectonema calothricoides. Data obtained (Figure 2) confirm the results shown by the polarographic method. Assays using Anabaena gave a negative result, even after 14 days of exposition. These results show that the different cyanobacteria assayed do not contribute to the removal of trichlorfon from the medium. They also show the rapid spontaneous degradation of the insecticide, already reported by other authors (Arthur and Casida 1957; Metcalf et al. 1959).

There are only a few reports of biodegradation of organophosphorus insecticides by cyanobacteria and they show different results. Nostoc linckia, Synechococcus elongatus and Phormidium tenue were capable of metabolizing monocrotophos and quinalphos (Megharaj et al. 1987) while Anacystis nidulans did not degrade parathion (Gregory et al. 1969) and Anabaena and Aulosira fertilissima did not degrade malathion (Rao and Lal 1987). To our knowledge this is the first report about the degradation of trichlorfon in relation with cyanobacteria. Other microorganisms such as the bacterium Pseudomonas, the $N_2$-fixing bacterium Rhizobium or the fungi Aspergillus, Penicillium and Fusarium biodegraded this insecticide (Lal 1982). And recently, biodegradation of trichlorfon by some green algae has been observed in our laboratory (Martinez, personal communication). The different role of cyanobacteria and green algae in the metabolism of trichlorfon seen in our laboratory differs from the work of Megharaj et al. (1987) where both categories of organisms were equally capable of degrading organophosphorus insecticides.

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