Some metabolic activities in the green alga *Scenedesmus bijuga* as affected by the insecticide trichlorfon

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Summary

This investigation studied the effects of trichlorfon on the growth and some physiological activities of the common freshwater alga *Scenedesmus bijuga* in the River Nile. The results showed that the cell number and Chl. *a* content of *Scenedesmus bijuga* decreased with increase in trichlorfon concentration. The data also showed that the total carbohydrate contents of *Scenedesmus bijuga* increased following treatment with low concentrations of trichlorfon (0.1 mM). All the applied treatments of trichlorfon strongly reduced the total protein content of *Scenedesmus bijuga* in comparison to control values. A gradual increase in total amino acids content was generally a function of trichlorfon concentration increase from 0.1 to 0.4 mM. As regards to nucleic acids the maximum level (0.58 and 1.60 mg g^{-1} DW of DNA and RNA, respectively) was obtained at 0.1 mM concentration of trichlorfon. However, any increase above this latter level was inhibitory. Trichlorfon treatment suppressed the activity of both acid and alkaline phosphatase in *Scenedesmus bijuga*. Consistently with this response, higher doses of the insecticides (0.4 and 0.8 mM) increased the glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT).

Key words: insecticides; metabolic activity; *Scenedesmus bijuga*; trichlorfon

Introduction

Pesticides are mainly synthetic organic compounds that are deliberately introduced into the environment to control selected organisms (Mellanby, 1978). These pesticides can have deleterious effects on algae by influencing soil algal growth, photosynthesis, nitrogen fixation, biochemical composition, and metabolic activities (Mishra and Pandey, 1989; Scarponi et al., 1991; Bhunia et al., 1991; El-Ayouty and Ezzat, 1991; Haroun et al., 1993; Mansour et al., 1993; Soliman et al., 1994; Fargasova, 1994; Eladel et al., 1999; Seguin et al., 2001; Ma and Liang, 2001; Ma et al., 2002; Mostafa and Helling, 2002; Friesen Pankratz et al., 2003). More specifically, orthophosphorus insecticides (malathiom, chlorpyrifos, fenitrothion, demeton and phorate) have been reported to reduce levels of cyano-bacterial pigments (Lal and Dhanaraji, 1985; Bhunia et al., 1991; El-Ayouty and Ezzat, 1991; Haroun et al., 1993; Mansour et al., 1993; Eladel et al., 1999; Sabater and Carrasco, 2001). Further investigation has focused on the effect of pesticides on nitrogen fixation in cyanobacteria (Soliman et al., 1994). On the other hand, Mishra and Pandey (1989) reported that the

addition of carbon sources, including glucose, acetate and some amino acids (glutamine, arginine, serine, tryptophan), enhanced resistance to thiobencarb toxicity in *Nostoc linckia.* Recently, Ma et al. (2002) have demonstrated that algae vary greatly in their responses to chemicals. Differential sensitivity of green protists to the compounds could induce species shifts within communities.

Trichlorfon is an organophosphate insecticide that is marketed under 22 different brand names, the most common being trichlorfon and chlorophos. It also has a drug name of metrifonate. It is used to control a large variety of pests including cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leaf miners and leaf-hoppers. It is frequently detected in surface run-off water from agricultural fields since it is used to protect vegetable, fruit and field crops. Trichlorfon is also used in the treatment of external parasites on fish and internal parasites in domestic animals. It is obvious from the number of uses of trichlorfon and its direct application and entry into the aquatic ecosystem that this pesticide is a potential threat to aquatic life. Like other organophosphate insecticides, trichlorfon is an acetylcholinesterase inhibitor that alters the behavior and physiology of the organism and can cause death. The chemical name for trichlorfon is dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate (Brecken-Folse et al., 1994). Molecular formula and the main structural characteristics of trichlorfon are are represented at Fig. 1 and in Table 1. Trichlorfon degrades rapidly (approximately 99% of applied trichlorfon degrades in 2 hours) in alkaline pond water (pH 8.5) at room temperature, but remains stable in the same pond water under acidic condition (pH 5.0) for two hours. The major product
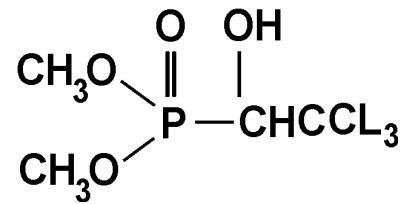

Fig. 1. Chemical structure of trichlorfon.

of degradation in pond water is dimethyl 2,2-dichlorovinyl phosphate and desmethyl DDVP (U.S. Environmental Protection Agency, 1978).

Pollution of water with several classes of pesticides will upset the natural balance among algal species as well as other microorganisms found in water bodies (Brock et al., 1995; Gustavson and Wangberg, 1995). The aim of this study was to examine the effects of trichlorfon on the growth and some physiological activities of the freshwater alga *Scenedesmus bijuga* that is common in the River Nile. A study of these effects may help to establish the maximum permissible concentration of this insecticide in the aquatic environment, since it is conceivable that even at low pollution levels it may affect the function and composition of natural aquatic systems.

Material and methods

ORGANISM AND CULTURE CONDITION

Scenedesmus bijuga (Turp.) Lagerh. was isolated from the River Nile at El-Minia (Egypt). Isolation and purification was made by dilution and plating technique.

Table 1. The main structural characteristics of trichlorfon.

Common name	Trichlorfon (BSI, ISO)
Chemical names	dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate
Common trade names	Anthon; BAY L 13/59; Bovinox; Briten; Cekufon; chlorofos (former USSR); Ciclosam; Danex; Denkaphon; Dipterex; Diptetes; Ditrifon; Equino-Acid; Leivasom; metrifonate; Neguvon; Proxol; trichlorfon; Trinex; Tugon
Chemical family	Organophosphorus; organochlorine
Molecular formula	$C_4H_8Cl_3O_4P$
Molecular weight	257.44
Physical form	Colourless crystals
Melting point	75-79°C
Boiling point	100°C at 0.1 mm Hg
Specific gravity	1.73 at 20°C
CAS registry number	52-68-6
Stability	Decomposition proceeds more rapidly with heating and above pH 6
Environmental effects	Surface water contamination
Solubility	In water at 20°C, 120g/l
Principle use	Insecticide

The alga was grown in 250-ml flasks containing 100 ml Kuhl's medium (Kuhl, 1962), and incubated in an illuminated incubator (Precision, USA) at 22°C and an irradiance of 150 μM m^{-2}s^{-1}, provided by cool white fluorescent lamps set on 14:10 h photoperiod. All cultures were shaken twice daily to prevent cells from clumping. Sterile technique was used at all times. After some introductory physiological studies on the growth behavior of *Scenedesmus biguja*, it was found that the optimum growth period was 10 days.

TREATMENTS

Trichlorfon (technical grade 95%) was obtained from KZ Company (Egypt). Concentrated trichlorfon (10 mM) was prepared in distilled water. Aliquots of the stock were added to culture flasks (250-ml). Medium was then added and flasks were left for one hour to obtain aqueous solutions of 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mM. Preliminary experiments were carried out to determine the suitable range of pesticide concentrations. All cultures (three per treatment) received identical inocula and were incubated under the prescribed growth conditions. To prevent the conversion of trichlorfon to dichlorvos (an even more toxic substance) at levels of alkalinity greater than pH 6.0, all the treatments and control were adjusted to pH 6.0 with NaOH and HCl. At the end of the incubation period cultures were filtered and washed several times by distilled water to measure the various experimental parameters. The values calculated are the mean of triplicates, and the standard deviation was less than 50% of these mean values.

PHOTOSYNTHETIC OXYGEN EVOLUTION

Photosynthetic oxygen evolution was measured at 27°C and an irradiance of 350 μM m^{-2}s^{-1} with a Clark type oxygen electrode. For these measurements the non-treated cultures were concentrated and an algal suspension containing 5% 10^8 cell ml^{-1} was used. Prior to the experiment the algae were resuspended in the trichlorfon-free incubation medium described above and the oxygen evolution was measured after the addition of trichlorfon concentrations. Before measurement, sodium dithionite was added to remove the oxygen down to zero value.

BIOCHEMICAL ANALYSIS

Cell number was determined using a Hemato-cytometer chamber. Chlorophyll *a* was estimated according to Metzner et al. (1965). The anthrone method (Roe, 1955) was applied for total carbohydrate estimation using fresh material and glucose as a standard. Total amino acid content was determined according to Moore and Stein (1948). Total protein was measured according to Lowry et al. (1951). Nucleic acids were extracted by the method of Shibko et al. (1967). DNA and RNA were colorimetrically determined at 600 and 670 nm using diphenyl amine and orcinol reagents, according to the methods of Burton (1968) and Ashwell (1957), respectively.

ALGAL EXTRACTION FOR ENZYME ASSAY

Fresh algal samples were instantly ground, accor-ding to Shabana and Khalil (1988), immediately after the experimental period with a known volume of distilled water and some pure acid washed sand. Samples were then centrifuged for 15 minutes, made up to a known volume and frozen. The methods of Bergmeyer (1974) were adopted for the estimation of both glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT). Both acid and alkaline phosphatases were estimated in the same extract by estimating the liberated phosphorous colorimetri-cally at 700 nm by the sulphite metol method (Burton and Riley, 1954).

STATISTICS

Results were tested by one-way analysis of variance (ANOVA). ANOVA effects and treatments differences were considered significant at $P<0.05$.

Results and discussion

Intensive crop production has used more and more pesticides over the past several decades. Increased production of crops, such as cereals, involves massive pesticides consumption that leads to significant contamination of surface water (Seguin et al., 2001). Tichlorfon constitutes the largest amount of all insecticides applied to agricultural land in Egypt. Trichlorfon contamination of surface waters as a result of agriculture non-point source inputs was identified as a major threat to aquatic ecosystems nationwide. Some uses of trichlorfon can result in its content in water exceeding 1.0 mM (Hammouda, 1994; Peters et al., 2001). Nowadays, increasing attention is given to the possible effects of pesticides on algae which play an important role in productivity and in gas exchange with the biosphere (Haroun et al., 1993; Battaglin and Fairchild, 2002; Friesen Pankratz et al., 2003).

The data illustrated in Fig. 2 clearly revealed that cell number of *Scenedesmus bijuga*, irrespective of some minor fluctuations, decreased with increased trichlor-fon concentration in the culture medium; the drop was more prominent and highly significant at larger than at

smaller doses. A similar inhibitory effect on cell number was reported for *Chlamydomonas* (Cain and Cain, 1984); *Scenedesmus obliqnus* and *Chlorella pyrenoidosa* (Ma et al., 2002); *Chlorella kesslerei* and *Anabaena inaequalis* (Mostafa and Helling, 2002). Fig. 2 further shows that the Chl. *a* content of *Scenedesmus bijuga* decreased continuously with increasing trichlorfon concentration. This reduction was significant at the highest trichlorfon concentration (1.0 mM). These findings are in agreement with several previously published data (Hammouda, 1994; Seguin et al., 2001; Mostafa and Helling, 2002). On the other hand, Haroun et al. (1993) reported that the content of pigments produced by *Scenedesmus quadriquda* was increased upon increasing asulam (herbicide) concentration.

The interference of trichlorfon with growth and photosynthetic Chl. *a* was further clarified by testing the effect of the insecticide on photosynthetic electron flow. When the inhibitory effect of trichlorfon on photosynthetic oxygen evolution was investigated, a remarkable degree of inhibition was observed with increasing concentration of the insecticide (Fig. 3). The highest inhibitory effect of trichlorfon on photosynthetic oxygen evolution (10% of the control value) was detected at the concentration of 1.00 mM. Marco et al. (1990) reported that the photosynthetic activity in *Anabaena* PCC 7119 was unaffected by trichlorfon treatment. However, a slight effect in *Gloeocapsa* sp. was detected after 24h of exposure. The inhibitory effect of trichlorfon on photosynthesis can be attributed to the alteration in photosynthetic electron transport by the insecticide binding to photosynthetic membranes (Lal and Dhanaraj, 1985).

The data presented in table 2 show that the total carbohydrate contents of *Scenedesmus bijuga* increased following treatment with lower concentrations of trichlorfon (0.1 mM). However, the amounts of these
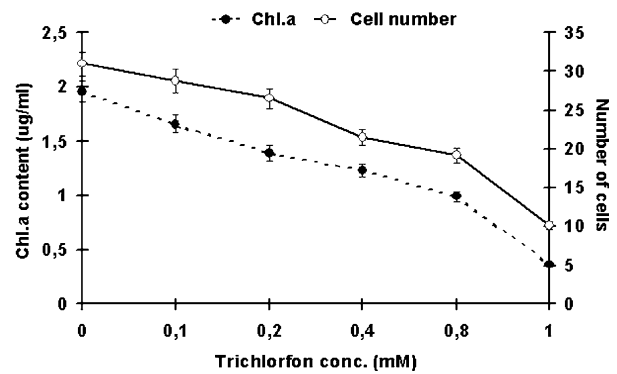

Fig. 2. Effect of trichlorfon on Chlorophyll *a* and cell number (No. x $10^7 l^{-1}$) of *Scenedesmus bijuga* at various concentration after 10 days growth period. Vertical bars indicate SE, n=3.

fractions appeared to be significantly decreased at the highest concentration of the insecticide tested. The slight increase in carbohydrate accumulation in *Scenedesmus bijuga* following treatment with small doses of trichlorfon compared with control could be due to the degradation of this chemical in the algae under consideration (Mansour et al., 1993). On the other hand, the inhibitory effect of relatively high concentrations of trichlorfon on carbohydrate production in the present study might be due to retardation in the rate of CO_2 photoassimilation (Mansour et al., 1993). The inhibition of total carbohydrate accumulation in *Tolypothrix* was recorded after treatment with atrazin (Shabana and Khalil, 1988). Similarly, Mansour et al. (1993) reported that relatively high concentration (3.5 ppm) of butachlor significantly decreased the levels of carbohydrate fraction of *Nostoc kihlmani*. However, Eladel et al. (1999) showed that no consistent changes depending on thiobencarb dose occurred in total carbohydrate content of *Protosiphon botryoides*.

Table 2. Total carbohydrates, total amino acids, total protein and nucleic acids of *Scenedesmus bijuga* on day 10 of trichlorfon treatment. Means (n=3). Results of one-way ANOVA comparison of treatments to controls indicate *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

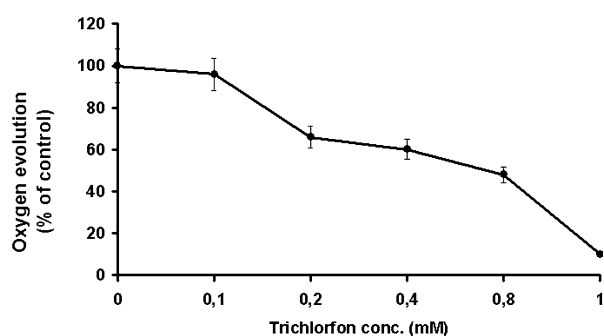| Trichlorfon conc.	Total carbohydrates	Total amino acids	Total protein	Nucleic acid mg g⁻¹ DW	
mM	mg g⁻¹ DW	mg g⁻¹ DW	mg g⁻¹ DW	DNA	RNA
0.0	507.00	10.12	20.12	0.56	1.41
0.1	519.34	18.60	18.00	0.58	1.60
0.2	482.00	24.81	17.77	0.54	1.44
0.4	463.67	20.55	11.78*	0.49	0.96
0.8	321.00**	7.07***	5.96**	0.32*	0.64*
1.0	210.00**	4.21**	1.84***	0.21*	0.28*
One-Way ANOVA	**	***	***	**	**

Fig. 3. Effect of different concentrations of tri-chlorfon on photosynthetic oxygen evolution in algal suspension from *Scenedesmus bijuga*. Vertical bars indicate SE, n=3.

The results of this investigation (table 2) further show that applying trichlorfon to *Scenedesmus bijuga* suppresses the total protein content in comparison to control values. This effect is more pronounced at higher doses. Eladel et al. (1999) reported that at 3 mg l^{-1} of thiobencarb the protein content of *Protosiphon botryoides* decreased. Although the mode(s) of action of pesticides are not well understood, they seem to inhibit fatty acids and protein synthesis (Tomlin, 1994; Eladel et al., 1999).

A gradual increase in total amino acids content was obtained when trichlorfon concentration increased from 0.1 to 0.4 mM, and the maximum value (24.81 mg g^{-1} DW) appeared at 0.2 mM (Table 2). Concentrations higher than 0.4 mM of trichlorfon were inhibitory. Higher concentrations of pesticides were inhibitory to total amino acids in *Nostoc muscorum*. In addition, the amino acids produced in algal cells differed quanti-tatively and qualitatively according to the type of alga and conditions of cultivation (El-Ayouty and Ezzat, 1991). Soliman et al. (1994) reported that the synthesis of some major amino acids depended on the provision of carbon skeleton from TCA cycle, which can be indirectly affected by the herbicide.

In the present investigation the maximum level of nucleic acids of *Scenedesmus bijuga* (0.58 and 1.60 mg g^{-1} DW of DNA and RNA, respectively) was obtained at 0.1 mM concentration of trichlorfon. However, any increase above this level was inhibitory (Table 2). El-Ayouty and Ezzat (1991) showed that DNA and RNA contents of *Nostoc muscorum* increased with the increase of prometryn concentrations until a maximum was reached at 3 ppm. Any increase above this value was inhibitory. Moreover, the view held by Scarponi with coauthors (1991) shows that the inhibitory effect of high concentrations of pesticide on protein or nucleic acids synthesis could be attributed to the blocking of L-histidine required for molecule synthesis.

The data of Fig. 4 show that trichlorfon treatment suppressed the activity of acid and alkaline phosphatase of *Scenedesmus bijuga*. Moreover, the highest doses of trichlorfon were more suppressive to the activity of these two enzymes. Concerning GOT and GPT enzymes, the lower doses of trichlorfon (0.1 and 0.2 mM) lead to decrease in the GOT and GPT activity of *Scenedesmus bijuga*. In contrast, higher doses of the insecticide (0.4 and 0.8 mM) caused an increase in the GOT and GPT activities. It is worth mentioning that at trichlorfon concentrations above 0.8 mM the enzyme activity was hardly inhibited (Fig. 5). The stimulation of GOT and GPT enzymes at relatively high doses of the herbicide used in the detoxification of pesticides, e.g., glutathine transferase (GST), implies that these enzymes are present in herbicides-resistant plants. They have been shown to catalyze the detoxification of several herbicides via their conjugation with the endogenous reduced glutathione (GSH) or homoglutathine hGSH (Scarponi et al., 1991). Mansour et al. (1993) reported that GOT and GPT activities of some Cyanophyta were markedly enhanced by different concentrations of butachlor, oxadiazon or thiobencarb. Soliman et al., (1994) revealed that the levels of malate dehydrogenase in *Nostoc kihlmani* and *Anabaena oscillarioides* increased significantly in response to treatment with low and moderate doses of pesticides, but was inhibited by higher doses. Furthermore, this study showed that higher concentrations of the herbicide suppressed the phosphatases and nitrogenase activity (Soliman et al., 1993).
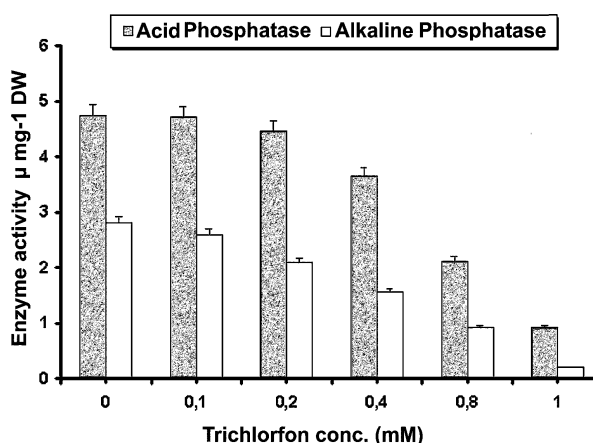
Acknowledgements

Fig. 4. Effect of different concentrations of trichlorfon on acid and alkaline phosphatase activity in *Scenedesmus bijuga* after 10 days growth period. Vertical bars indicate SE, n=3.
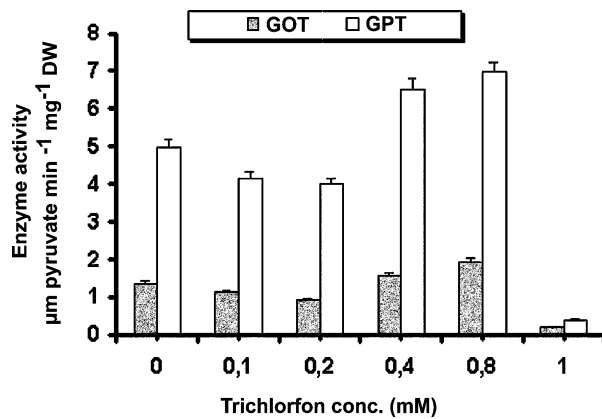
Fig. 5. Effect of different concentrations of trichlorfon on GOT and GPT activity in *Scenedesmus bijuga* after 10 days growth period. Vertical bars indicate SE, n=3.

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