Bull. Environ. Contam. Toxicol. (2005) 74:1192–1198

DOI: 10.1007/s00128-005-0707-y

Bulletin of Environmental Contamination and Toxicology

# Toxicity of Three Insecticides in a Standard Algal Growth Inhibition Test with *Scenedesmus subspicatus*

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Received: 19 November 2004/Accepted: 11 March 2005

Modern industry and agriculture have been releasing large quantities of different chemicals, among them insecticides, into the environment. They disturb water ecosystem balance by direct toxic effects on plants and animals and also by their ability for bioaccumulation and transfer in food chains. Planktonic algae, as primary producers, play an essential role in the water environment. Since they are the basic link in the aquatic food chains and they are a key functional group of organisms, they are of fundamental importance in proper structure and function of the whole ecosystem. Moreover, planktonic algae are sensitive indicators for testing different effects of substances discharged into the water. Therefore, the investigations on the influence of xenobiotics on unicellular algae have been generally accepted.

Fenitrothion, deltamethrin and bensultap are insecticides commonly used in agriculture. They have replaced extremely harmful organochlorine compounds because of their shorter perisitence and lower toxicity to non-target organisms. Since they are widely applied in pest control, they could be potential sources of water pollution. Extensive literature is available on the effects of insecticides on target organisms and non-target groups such as bacteria, cyanobacteria, fungi and invertebrates. However, there are not many studies on microalgae. Significant growth suppression followed treatment of different phytoplankton species with fenitrothion (Kent and Weinberger 1991; Kent and Caux 1995; Kent and Currie 1995; Sabater and Carrasco 2001). Simultaneously with the inhibition of growth, chlorophyll *a* content and photosynthesis intensity in *Chlamydomonas reinhardtii* were decreased (Wong and Chang 1988). However, at lower concentrations stimulating effects were observed. Ferrando et al. (1996) found that in *Nannochloris oculata* cultures fenitrothion caused a marked decrease in biomass and cell division.

Deltamethrin belongs to pyrethroid insecticides, a group of chemicals that is relatively non-persistent and does not accumulate in the water environment (Smith and Stratton 1986). However, the review of Johri et al. (1997) suggests that pyrethroids could adversely affect aquatic algae and other microorganisms. Baeza-Squiban et al. (1987) showed that the growth of *Dunaliella* and *Chlamydomonas* was significantly slower with deltamethrin. In spite of rapid disappearance of deltamethrin from the

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aquatic environment, its considerable effects on phytoplankton communities could be observed (Caquet et al. 1992). Because of the data shortage on the toxicity of organophosphorus and pyrethroid insecticides towards green unicellular algae and the lack of information on the effects of bensultap, a nereistoxin analogue, the influence of three commonly used insecticides on *Scenedesmus subspicatus* 86.81. SAG was studied in a freshwater algal growth inhibition test.

## MATERIALS AND METHODS

The green alga Scenedesmus subspicatus 86.81 SAG was used as the test organism and was obtained from the Culture Collection of Algae, University of Texas, Austin (UTEX). The strain is a widely accepted bioindicator used in water pollution research. The stock algal culture was maintained on agar slates supplemented with bactopeptone and BBM medium (Bold 1949) under the following conditions: ca. 6 $\mu$molphotons $m^{-2}$ $s^{-1}$ from 13 W cool-white fluorescent tubes (Philips); lighting cycle, 12 h light followed by 12 h darkness; temperature 20 ± 1 °C.

The following insecticides were used: fenitrothion, 3-methyl-4-nitrophenyl phosphothionate ("Organika-Azot" Chemical Co., Poland); deltamethrin, S-alpha-cyano-3-phenoxybenzyl-1R-cis-3-2,2-dibromovinyl-2,2-dimethylocyclopropane carboxylate (Roussel Uclaf, France) and bensultap, S,S'-2-dimethylaminotrimethylene di(benzene-thiosulphonate) ("Fregata" Chemical Co., Poland). Stock solutions of analytical grade chemicals were prepared immediately before use.

Three days before the test was to start the preculture was set up. Inoculum, ca. 10000 cells mL, was added to 50 mL of culture medium (International Standard ISO 8692, 1989). pH of the medium was adjusted to 8.3 ± 0.2. The preculture was incubated at 22 ± 2 °C, under continuous illumination from 20 W cool-white fluorescent Philips tubes. The intensity of photosynthetic active radiation (PAR) was about 80 $\mu$molphotons $m^{-2}$ $s^{-1}$. After three days the preculture was in the exponential growth phase. Several preliminary experiments were performed to determine the concentration ranges of insecticides causing ca. 10-90% growth reduction in relation to the control cultures. The concentrations were set up at geometric progression and produced 4-5 levels of effects. The following concentrations were applied: fenitrothion – 0.25, 0.5, 1 and 2 mg/L; deltamethrin – 0.63, 1.25, 2.5, 5 and 10 mg/L; bensultap – 0.5, 1, 2, 4, 8 and 16 mg/L. Initial cell density and culture conditions were the same as in the preculture. The test vessels were shaken twice a day and test duration was 72 hr. Cell density in the cultures were determined every 24 hr with a Bürker hemacytometer. Following the standard protocol of ISO 8692 (International Standard ISO 8692, 1989), the values of growth rate (r) were calculated for all sets. The data on cell number and growth rate were compared to the appropriate controls (0 mg/L) by t-test. $EC_{50}$ (0-72 hr) values and 95% Confidence Limits (CLs) were determined based on the linear regression of percentage growth inhibition on log dose of insecticides (Sokal and Rohlf 1981). In the present paper, $EC_{50}$ values, i.e. calculated concentrations that would inhibit growth by 50% as compared to the

control treatment, were estimated based on growth curve area ($E_bC_{50}$) and growth rate ($E_rC_{50}$). Data in figures and tables are the means and standard deviations from at least three independent tests with triplicate cultures and samples within each individual experiment. The statistical analyses were performed using the commercial software packages Microsoft Excel 5.0.

## RESULTS AND DISCUSSION

The effects of three insecticides on the growth of *S. subspicatus* are shown in Fig. 1. The data compared by t-test revealed that the lowest concentrations of the substances did not produce statistically significant inhibition in relation to the control cultures. Similarly as in the previous studies (Burkiewicz and Makuch 2001), fenitrothion appeared to be the compound of very strong toxic influence. A marked reduction in cell number was found after 48 hr of incubation at 2 mg/L. After 72 hr the range of inhibiting concentrations was 0.5-2 mg/L. The higher the concentration used, the greater the toxic effect. The results obtained are in agreement with those of Kent and Weinberger (1991) who observed the algistatic effect of fenitrothion (1 and 10 mg/L) on *S. obliquus* growth after 7-day exposure. Kent and Caux (1995) also found a reduction in the final standing crop of *Ankistrodesmus falcatus, Chlamydomonas reinhardtii* and *S. obliquus* in 96-hr algal growth assays at 1 and 10 mg/L of fenitrothion. Fenitrothion concentrations higher than 1 mg/L markedly reduced *N. oculata* cell densities and biomass after 72 hr exposure (Ferrando et al. 1996). The results of Sabater and Carrasco (2001) indicated that within five species examined *S. acutus* and *S. subspicatus* appeared to be the most sensitive to fenitrothion. However, in long-term cultures (14 days) of twelve freshwater phytoplankton species (Kent and Currie 1995) the inhibiting effects of fenitrothion were observed mainly at the highest concentrations tested, i.e. 10 mg/L. Deltamethrin was decidedly less toxic to the algal cultures as compared to fenitrothion. At 2.5, 5 and 10 mg/L the cell divisions were significantly reduced after 24 hr of treatment resulting in a slower growth (Fig. 1). After 48 and 72 hr a decrease in population density was noted also at 1.25 mg/L. The extent of cell multiplication and consequently the growth of *S. subspicatus* was a function of the concentration of the insecticide in the medium and time of exposure. Despite the rapid disappearance of deltamethrin from the water (Thybaud 1990, Caquet et al. 1992), the compound could adversely affect algal growth. This was demonstrated by Baeza-Squiban et al. (1987) who found that the commercial formulations of deltamethrin (DECIS, DECIS EC and DECIS FLO) inhibited cell proliferation of a marine alga, *Dunaliella bioculata*. Growth was inhibited over a concentration range of $5 \times 10^{-6}$ to $9 \times 10^{-6}$ M. However, the authors did not notice any growth or morphology modifications of algal cells under the influence of pure deltamethrin. Therefore, they attributed the toxic effects to the matrix used in the commercial formulations. The results obtained in experiments with bensultap also indicate a less harmful influence than that of fenitrothion. After 24 hr of incubation a marked decrease in cell number was found at 2-16 mg/L. With the passage of time (48 and 72 hr) the range of reducing concentrations increased and was 1-16 mg/L. The extent of growth response of the alga depended on the level of bensultap in the cultures.

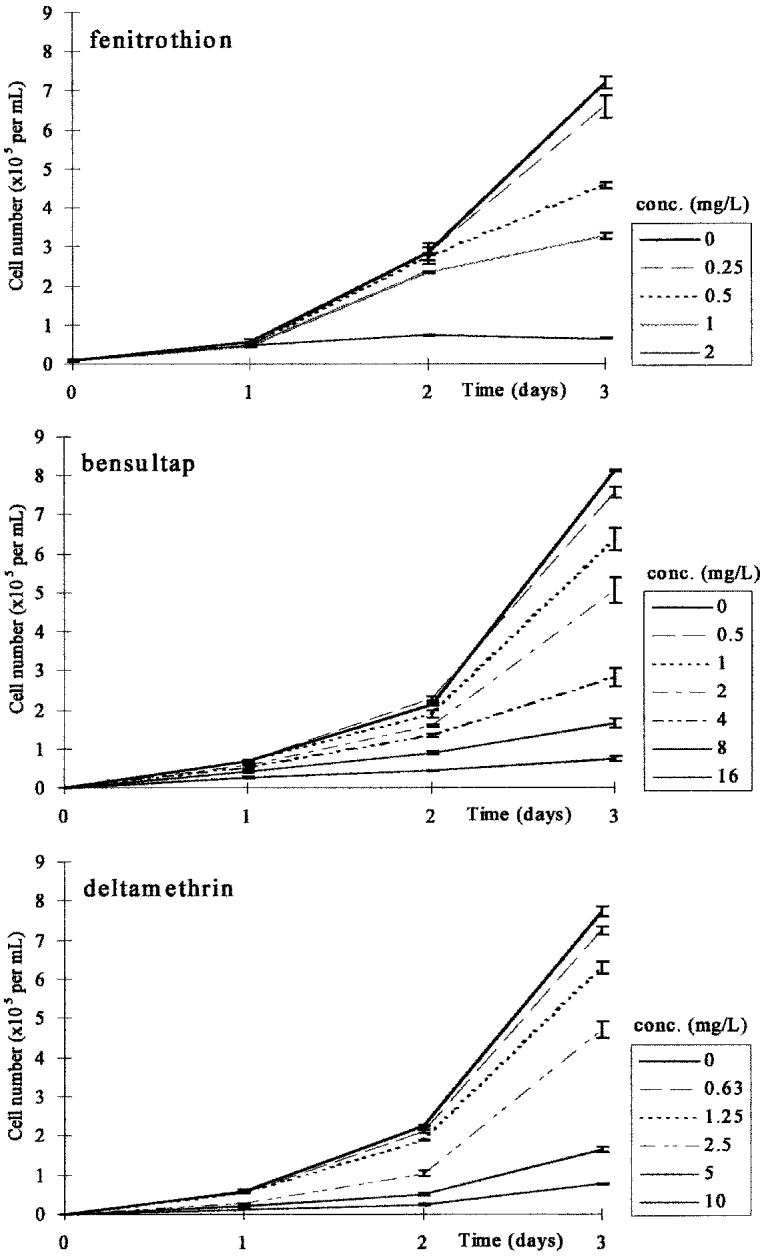


**Figure 1.** The influence of three insecticides on *S. subspicatus* growth. Data are expressed as a mean. Error bars refer to the standard deviations for 3 replicated experiments.

**Table 1.** Growth rate (days$^{-1}$) of the alga *S. subspicatus* when exposed to various insecticide concentrations for 72 hr.

| Insecticide | Concentration (mg/L) | Growth rate (days$^{-1}$) |
|---|---|---|
| Fenitrothion | 0 | 1.50 ± 0.13 |
| | 0.25 | 1.51 ± 0.10 |
| | 0.5 | 1.42 ± 0.09 |
| | 1 | 1.35 ± 0.11* |
| | 2 | 0.72 ± 0.07* |
| Deltamethrin | 0 | 1.52 ± 0.13 |
| | 0.63 | 1.43 ± 0.16 |
| | 1.25 | 1.42 ± 0.14 |
| | 2.5 | 1.28 ± 0.10* |
| | 5 | 1.01 ± 0.06* |
| | 10 | 0.72 ± 0.02* |
| Bensultap | 0 | 1.50 ± 0.15 |
| | 0.5 | 1.52 ± 0.12 |
| | 1 | 1.46 ± 0.06 |
| | 2 | 1.34 ± 0.10* |
| | 4 | 1.15 ± 0.09* |
| | 8 | 1.05 ± 0.09* |
| | 16 | 0.74 ± 0.06* |

Values are means ± SD

* Significantly different from control ($P < 0.05$)

Over the exposure duration, growth reduction in different degrees, from a slight one at lower insecticide concentrations to a drastic one at higher levels suggest the sensitivity and susceptibility of *S. subspicatus* to various chemicals. Fenitrothion was found to be the most harmful insecticide whereas deltamethrin and bensultap inhibited alga growth to a lesser extent.

Table 1 shows the growth rates of algal populations treated with various insecticide concentrations. At low concentrations the compounds tested did not affect r values. The growth rates were significantly reduced by higher concentrations, i.e. 1 and 2 mg/L of fenitrothion, 2.5-10 mg/L of deltamethrin and 2-16 mg/L of bensultap. Fenitrothion showed the largest inhibitory influence among the insecticides tested. Unfortunately, in the literature there is a lack of data on the effects of deltamethrin and bensultap on algal growth rates. However, the results obtained with fenitrothion are in accordance with the other authors who found a decrease in growth rate with an increase in fenitrothion concentration (Wong and Chang 1988; Kent and Caux 1995; Kent and Currie 1995; Ferrando et al. 1996; Sabater and Carrasco 2001).

Table 2 presents the $E_bC_{50}$ values when *S. subspicatus* was exposed to the insecticides in 72 hr growth tests. The alga was the most sensitive to fenitrothion. In the

**Table 2.** The $EC_{50}$ (0-72 hr) values and associated 95% Confidence Limits (CLs) for three insecticides. The values were determined based on growth curve area.

| Insecticide | $E_bC_{50}$ (mg/L) | 95% Confidence Limits (mg/L) | |
|---|---|---|---|
| | | Lower | Upper |
| Fenitrothion | 1.03 | 0.29 | 6.49 |
| Deltamethrin | 2.56 | 1.44 | 4.59 |
| Bensultap | 3.37 | 2.60 | 5.39 |

case of deltamethrin and bensultap $EC_{50}$ values were higher, 2.5 and 3.6 times, respectively. The growth reduction in fenitrothion treated cultures was between 6 and 81% in relation to the control cultures. However, the broad CLs range for fenitrothion was obtained and the upper CL differed considerably from the highest concentration used. It could be probably avoided by applying more fenitrothion concentrations. The calculated values of $E_rC_{50}$ (derived from growth rate) were 2.08, 9.85 and 15.11 mg/L for fenitrothion, deltamethrin and bensultap, respectively. Since the values were very close to the highest concentrations used, CLs were not calculated.

Kent and Currie (1995) found that 12 tested species differed significantly in their sensitivity to fenitrothion, as $EC_{50}$ values for 96 hr growth ranged from 0.8 mg/L (*Staurastrum* sp.) to 24 mg/L (*Chlorella vulgaris*). In a study with permethrin, Smith and Stratton (1986) found that $EC_{50}$ values for *C. pyrenoidosa* and *S. quadricauda* were higher (> 10 mg/L) than those of its degradation products (1.4-8 mg/L).

The present study showed that fenitrothion was the most toxic insecticide whereas bensultap and deltamethrin appeared to be less harmful substances. The inhibition effects depended on the concentration of the compounds examined and the period of their influence on algal cells. Considering the lack of data on the toxicity of insecticides, especially deltamethrin and bensultap, towards unicellular algae and the common use of these substances, it is necessary to investigate their effects further.

*Acknowledgments.* Thanks are due to Barbara Janas for technical assistance. The study was supported by grant from University of Gdańsk (BW 1110-5-0244-4).

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