African Journal of Biotechnology Vol.5 (1), pp. 045-048, 5 January 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Toxicity evaluation of flucycloxuron and diflubenzuron on the cellular model, *Paramecium* sp.

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Accepted 11 November, 2005

The effect of two pesticides, the diflubenzuron (DFB) and flucycloxuron (FCX) was studied on the ciliated protiste cellular model, *Paramecium* sp. Treatment with the DFB at 10 and 20 µg/ml reduces the growth of this protiste appreciably. The survey of the respiratory metabolism by the polarography technique (oxygen electrode) shows a sensitive inhibition of the oxygen consumption. In the case of the FCX, treatment with the two concentrations (10 and 20 µg/ml) reveals an inhibition of the ciliated protiste growth. This pesticide also inhibits the respiratory metabolism of ciliated protiste. This effect is a lot more marked with the FCX that with the DFB.

Key words: *Paramecium* sp., flucycloxuron, diflubenzuron, pesticide, respiratory metabolism.

INTRODUCTION

Flucycloxuron or andalin (1 - [- (4chloro -α- cyclopropyl benzyldeaminp-oxy) p-to yl]-3-(2, 6-difluorobenzoyl) urea) is a molecule with insecticide and acaricide activities (Scheltes and al., 1988). it affects the molting cycle of Tenebrio molitor (Soltani et al., 1993) and reduced the thickness of the cuticle (Soltani et al., 1996). This pesticide is an insect growth regulator and acts on molting of the caterpillars.

Diflubenzuron or dimilin (1-chlorophenyl)-3-(2,6-difluorobenzoyl) urea) is a pesticide of third generation with a large specter of activity. It is used mainly in agriculture; forests and cereal farms to control insects. It is considered a poison when contacted and ingested. This pesticide inhibits the synthesis of the chitin and interferes with the formation of the insects' cuticle (exoskeleton), probably by the inhibition of the N-acetylglucosamine incorporation in the chitin (Nakagawa et al., 1993). It has been shown that the diflubenzuron could modify the metabolism of the molting hormones (Yu and Terriere, 1977; Soltani et al., 1984).

The present study reports the effect of these two pesticides on the growth, morphology and respiration a Paramecium sp., which is considered as excellent cellular model.

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MATERIALS AND METHODS

Biologic material and culture techniques

The microorganism, Paramecium sp., is chosen because of its cuticule comparable to insects' cuticule and its simple culture has been described by Wichterman (1953). The two pesticides are diluted in acetone in concentrations of 1, 10 and 20 µg/ml.

The addition of insecticides in the culture middle is done before the apparition of the cells. The kinetics of growth is followed every day by the measure of the optic density (600 nm) (Lavergne, 1986). The polarography technique (Djebar and Djebar, 2000) permits the measurement of the respiratory metabolism of the cells every day.

The neutral red coloration technique according to the method of Ovenbird (1990) modified by Rouabhi (2002) was used.

RESULTS

Effect of flucycloxuron on Paramecium sp.

Flucycloxuron at 1 µg/ml do not have any effect on the growth of the paramecia. But in a concentration of 20 µg/ml, it inhibits the growth (about 50%) (Figure1). It also

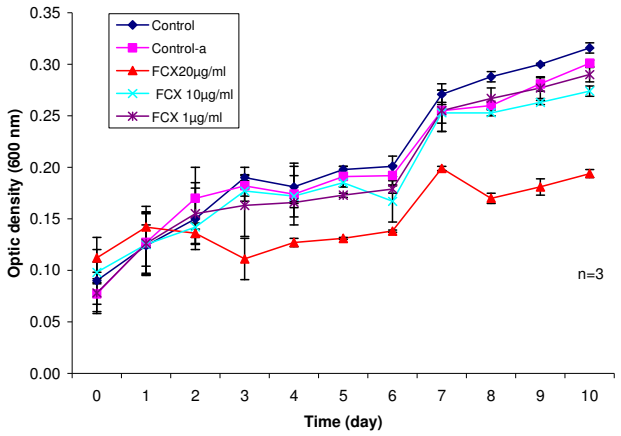

Figure 1. Effect of different concentrations of flucycloxuron on *Paramecium* sp. growth.

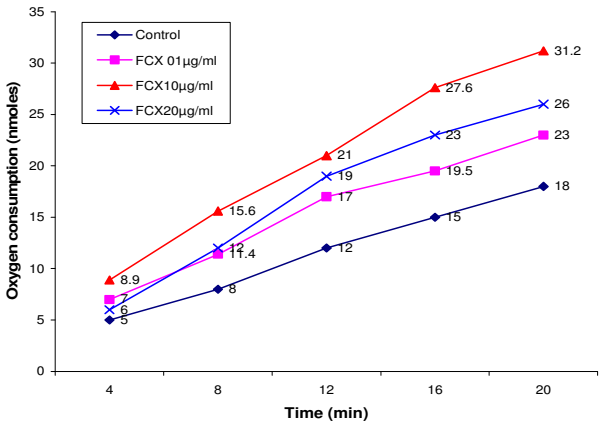

Figure 2. Effect of different concentrations of flucycloxuron on respiratory metabolism of *Paramecium* sp.

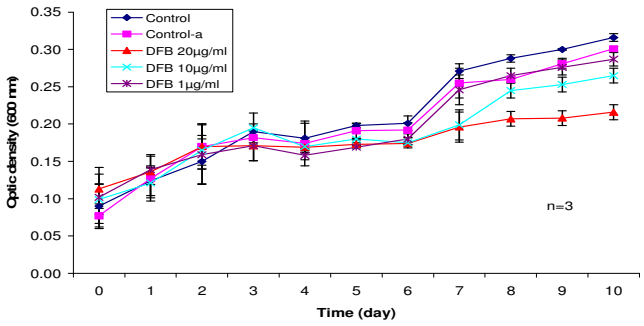

Figure 3. Effect of different concentrations of Diflubenzuron on *Paramecium* sp. growth.

leads to a stimulation of oxygen consumption in all studied concentrations (Figure 2).

Effect of the Diflubenzuron on Paramecium sp:

Diflubenzuron seems to disturb the microorganism growth in dose-dependent manner. The effect is more important at 20 µg/ml concentration (about 50%) (Figure 3). It is necessary to note that diflubenzuron in the concentration of 20 µg/ml stimulates oxygen consumption (26 nM O2) (Figure 4).

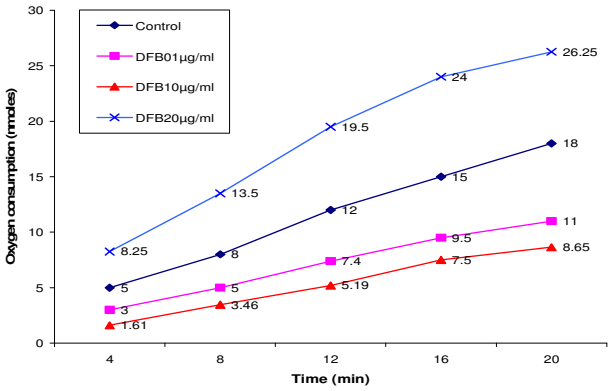

Figure 4. Effect of different concentrations of Diflubenzuron on respiratory metabolism of *Paramecium* sp.

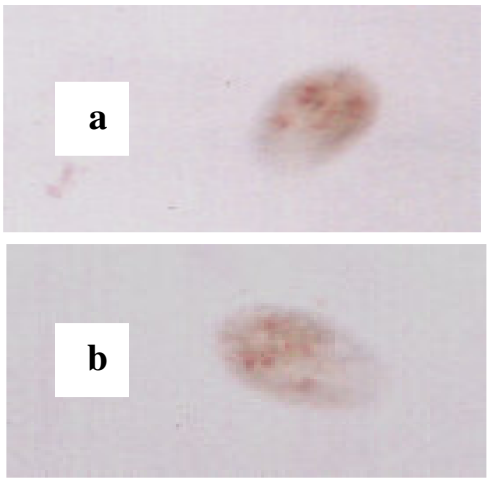

Figure 5. Paramecia observed with photonic microscope, Objective (x10). **a:** Paramecia control. **b:** Paramecia control-acetone.

Coloration with neutral red

The coloration technique allows us to estimate the effect of insecticides on the paramecia cuticule. The paramecium control and control-acetone appear with a diffused pink coloration, ovoid form and swim in regular trajectory (Figures 5a and b). The paramecia treated by the diflubenzuron at the three concentrations (Figures 6a,

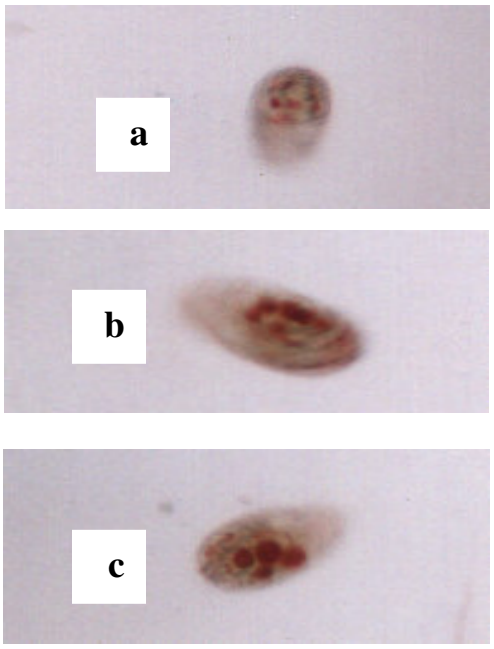

Figure 6. Paramecia observed with photonic microscope, Objective (x10). **a:** Paramecia treated with 1 µg/ml of Diflubenzuron. **b:** Paramecia treated with 10 µg/ml of Diflubenzuron. **c:** Paramecia treated with 20 µg/ml of Diflubenzuron.

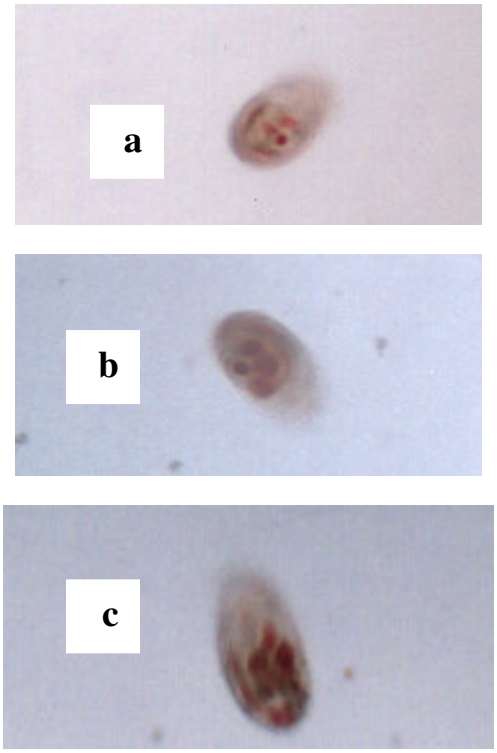

Figure 7. Paramecia observed with photonic microscope, Objective (x10). **a:** Paramecia treated with 1 µg/ml of Flucycloxuron. **b:** Paramecia treated with 10 µg/ml of Flucycloxuron. **c:** Paramecia treated with 20 µg/ml of Flucycloxuron.

b and c), present a darker coloration localized well to the vacuoles level, and the coloration intensity increases with the concentration of the pesticides.

In the case of the paramecia treated in the three concentrations of the flucycloxuron (Figures 7a, b and c), the cells appear with a coloration more marked, notably in the border and in the vacuoles.

The coloration by the neutral red permits better visualize the effect of the flucycloxuron and the diflubenzuron on the thickness and the integrity of the paramecia cuticule.

DISCUSSION

It is known that microorganisms represent one of the links of which pesticides could be transmitted through the food chain to man. In present work we try to evaluate a possible toxicity of the diflubenzuron and the flucycloxuron on an organism other that the target insects. Because of the absence of relative data on the environmental toxicity of these two pesticides, we chose the cellular model Paramecium sp. to for evaluation.

The presence of the halogen group on the two molecules (diflubenzuron and flucycloxuron) makes them very reactive (Marbury and Crosby, 1996). Klitschka et al. (1986) reported on the irreversible long-term effect of the diflubenzuron because its incorporation to the RNA. The impact of the diflubenzuron and the flucycloxuron on the environment is revealed by a test of cytotoxicity that we developed in the laboratory. Diflubenzuron as well as the flucycloxuron inhibits the growth of this ciliated protiste. This inhibition of the growth is even more pronounced at elevated concentrations (10 and 20 µg/ml).

This work shows that the diflubenzuron would accumulate in Paramecium sp. It has been observed that diflubenzuron acts on the development and the reproduction of Tenebrio molitor (Soltani et al., 1995, 1996). Flucycloxuron is active on the insects by interfering with the formation of the cuticle via the chitin synthesis (Soltani et al., 1993).

Finally, concerning the respiratory metabolism of the studied micro-organism, our results reveal an effect more marked in the flucycloxuron (especially for the highest concentration) treatment than that of diflubenzuron, which reinforces the results on growth inhibition. The result of the neutral red coloration technique indicates a reduction in the thickness of the cuticule confirming the results of Soltani et al. (1995) on the cuticle of T. molitor.

In conclusion the ciliated protistes are excellent models for evaluation of the xenobiotic toxicity especially in the environmental domain.

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