

Full Length Research Paper

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

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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 than with the DFB.

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

INTRODUCTION

Flucycloxuron or andalin (1 - [-(4chloro - - 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 reduces 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.

MATERIALS AND METHODS

Biologic material and culture techniques

The microorganism, *Paramecium* sp., is chosen because of its cuticle comparable to insects' cuticle 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 (Djebbar and Djebbar, 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

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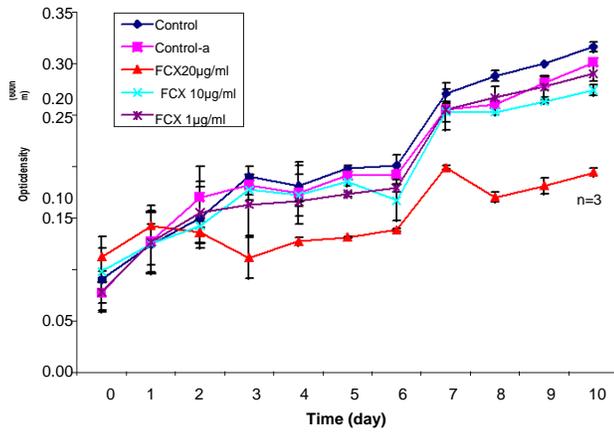


Figure 1. Effect of different concentrations of flucyclozuron on *Paramecium sp.* growth.

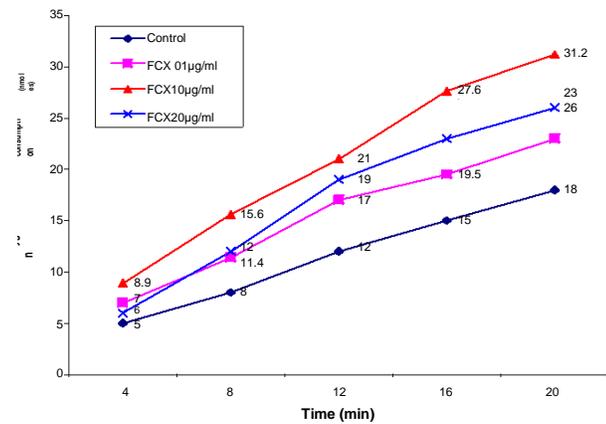


Figure 2. Effect of different concentrations of flucyclozuron on respiratory metabolism of *Paramecium sp.*

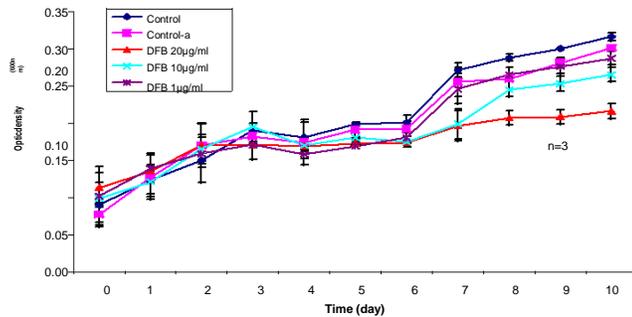


Figure 3. Effect of different concentrations of Diflubenzuron on *Paramecium sp.* growth.

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).

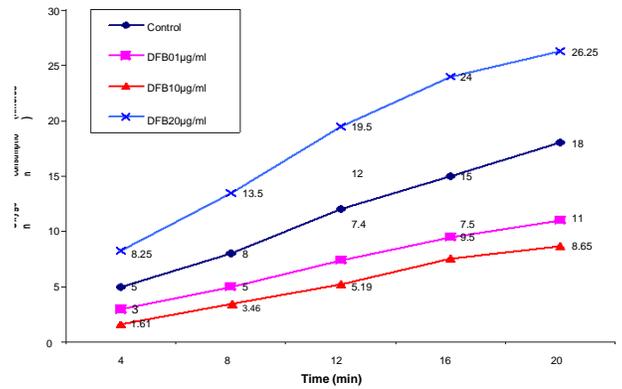


Figure 4. Effect of different concentrations of Diflubenzuron on respiratory metabolism of *Paramecium sp.*

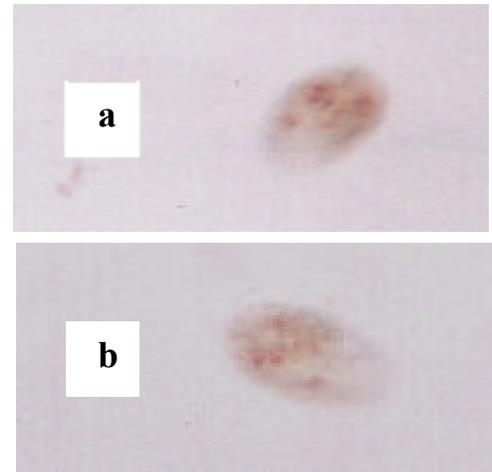


Figure 5. Paramecia observed with photonic microscope, Objective (x10) . **a:** Paramecia control. **b:** Paramecia control-acetone.

Coloration with neutral red

The coloration technique allows us to estimate the effect of insecticides on the paramecia cuticle. 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,

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

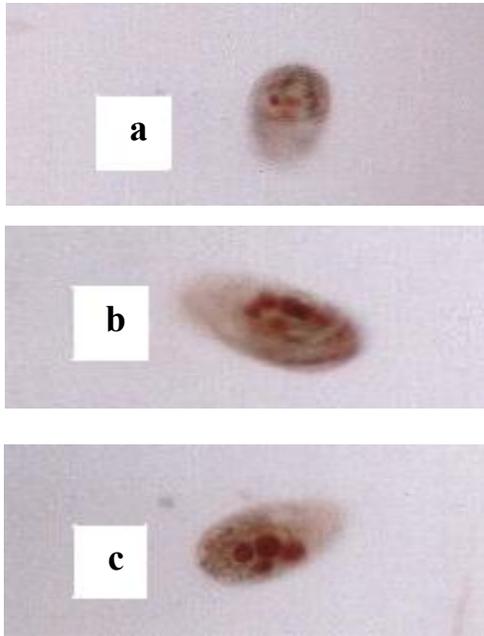


Figure 6. Paramecia observed with photonic microscope, Objective (x10). **a:** Paramecia treated with 1 µg/ml of Diflubenzuron. **b:** Paramecia treated with 10 µg/ml of Diflubenzuron. **c:** Paramecia treated with 20 µg/ml of Diflubenzuron.

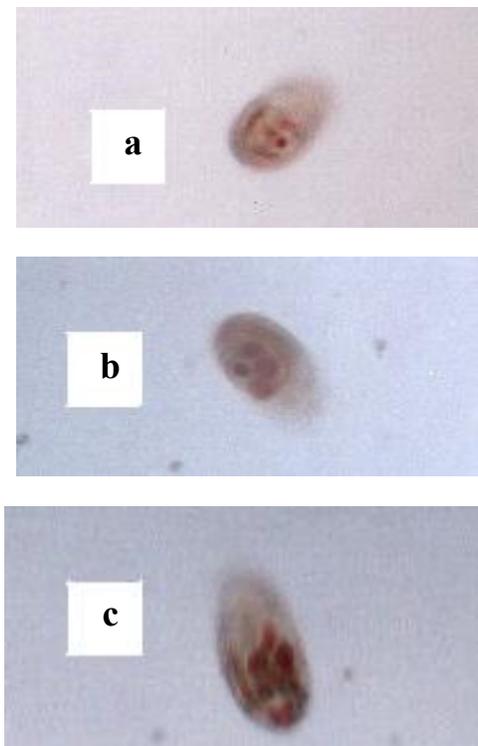


Figure 7. Paramecia observed with photonic microscope, Objective (x10). **a:** Paramecia treated with 1 µg/ml of Flucyclohexuron. **b:** Paramecia treated with 10 µg/ml of Flucyclohexuron. **c:** Paramecia treated with 20 µg/ml of Flucyclohexuron.

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 flucyclohexuron (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 flucyclohexuron 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 flucyclohexuron on an organism other than 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 flucyclohexuron) 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 flucyclohexuron on the environment is revealed by a test of cytotoxicity that we developed in the laboratory. Diflubenzuron as well as the flucyclohexuron 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). Flucyclohexuron 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 flucyclohexuron (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 cuticle 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.

REFERENCES

- Djebar MR, Djebar H (2000). Bioénergétique, les mitochondries végétales. Revue des Sciences et Technologies. Synthèse; Publication de l'Université de Annaba; Ed. Vegator: 103 pages.

- Klitschka GE, Mayer RT, Droleskey RE, Norman JO, Chen AC (1986). Effects of chitin synthesis inhibitors on incorporation of nucleosides into DNA and RNA in a cell line from *Manduca sexta* (L.). *Toxicology* 39: 307-315.
- Lavergne M (1985). Etude des effets du Diazépam et du Medazepam sur un protiste cilié. Mémoire de D.E.A., de l'Université de Paris VII 46 pages.
- Marbury AS, Grosby GD (1996). Fate and disposition of Diflubenzuron in Rice fields. *Environmental Toxicology and Chemistry*. University of California, Davis, California 95616. USA, V: 15, 11: 1908-1913.
- Nakagawa Y, Matsunra F, Hashino. Y (1993). Effect of DFB on incorporation of [3H]-N-acetyl glucosamine ([3H]NAGA) into chitin in the intact integument from the newly molted American cockroach *Periplaneta americana*. *Comp. Biochem. Phys.* 106: 711-715.
- Rouabhi R (2002). Toxicité de deux pesticides : le Flucycloxuron et le Diflubenzuron sur deux modeles cellulaires *Paramecium.sp* et *Tetraselmis suecica*. Mémoire de Magister en Physiologie appliquée, option :Ecotoxicologie animale. Université de Annaba: 81 pages.
- Scheltes P, Hoffmann TW, Groussourt AC (1988). Field Data on PH.70-23 a novel benzoylphenylurea controlling mills and insects in arrange of crops. Brught crop protection conference. Pests and diseases, pp. 559-566.
- Soltani N, Delbecque JP, Delachambre J, Mauchamp B (1984). Inhibition of ecdysteroid increase by Diflubenzuron in *Tenebrio molitor* pupae and compensation of Diflubenzuron effect on cuticule secretion by 20-hydroxyecdysone. *Intern. J. Invert. Reprod. Develop.* 7: 323-332.
- Soltani N, Chebira S, Delbecque J, Delachambre J, (1993). Biological activity of Flucycloxuron, a novel benzoylphenylurea derivate, on *Tenebrio molitor*: comparison with Diflubenzuron and Tiflumuron, *Experientia* 49 (12):1088-1901.
- Soltani N, Chebira S, Pitoizet N, Delbecque J.P, Delachambre J (1995). Effect of Flucycloxuron, a novel benzoylphenylurea derivate, on the in vivo and in vitro, production of ecdysteroids in *Tenebrio molitor*. *Med. Fac. Landbowi. Univ. Gent.* 607 (3b):1017-1022.
- Soltani N, Soltani-Mazouni N, Quennedey B, Delachambre J (1996). Protein synthesis in developing ovaries of mealworm under in vivo and in vitro conditions: effects of Diflubezuron. *J. Stored Prod. Res.* 32 3: 205-212.