

Growth, Chitin and Respiratory Metabolism of *Tetrahymena pyriformis* Exposed to the Insecticide Novaluron

¹Rouabhi Rachid, ²H. Djebar-Berrebbah and ²M.R. Djebar

¹Nature and life Sciences Institute, Biology Department, Tebessa University Center, 12000, Algeria

²Laboratory of Cellular Toxicology, Annaba University, 23000, Algeria

Abstract: The struggle against the agricultural ravagers was increased in few last years; many products were appeared and destined to kill the harmful insects, of which we did not know their effects on ecosystem and non-target animals. In present work, we try to investigate the ecotoxicological effects of Novaluron; an Insect growth Regulator (IGR) pesticide, on ecosystem and biomasses by the estimation of the growth, chitin and respiratory metabolism of *Tetrahymena pyriformis* as a non-target aquatic organism exposed to this pesticide. The pesticide was tested *in vitro* by the addition of three concentrations (1, 10 and 20 µg/ml) in strictly controlled conditions, to the culture medium before/after apparition of the protozoan. *Tetrahymena* population growth was evaluated by optic density at 600nm method, for the cells number we used the account under an optical microscope, the generation time and number were evaluated, also we evaluated the respiration of *Tetrahymena* population by oxygen electrode. Treatment with 1, 10 and 20 µg/ml of the pesticide affects the growth (proliferation) of *T. pyriformis* in concentration-dependent manner, also it increases the generation time and response percentage in concentration-dependent manner. The respiratory metabolism of protozoan is perturbed with the treatment by Novaluron at three concentrations, noting that the oxygen consumption was increased at 10 µg/ml, contrarily to the other two concentrations, which they had an inhibition effect especially for 20 µg/ml of pesticide. The effect on chitin synthesis was followed by neutral red coloration method; the effect on chitin is translated by the quantity and speed of the neutral red penetration. The results showed an alteration of the chitin integrity in concentration-dependent manner. All data showed an ecotoxicological effects on this protozoa, this perturbation might be transferred to the higher organisms by bioaccumulation in trophic chain.

Key words: Pesticides % IGR % Chitin synthesis inhibitors % Cytotoxic % *Tetrahymena pyriformis* % Respiratory metabolism

INTRODUCTION

Many of the pesticides used in the modern agriculture have the potential to influence the number and functions of a diverse range of water and soil microorganisms [1]. Benzoylureas are an entirely different class of insecticides that act as insect growth regulators (IGRs). More than being the typical poisons that attack the insect nervous system, they interfere with chitin synthesis and they are more taken up by ingestion than by contact [2]. Their value appeared in the control of caterpillars, [3, 4]. The benzoylureas act on the larval stages of most insects by inhibiting or blocking the synthesis of chitin [2], a vital and almost indestructible

part of the insect exoskeleton [5]. Typical effects on developing larvae are the cleaving of malformed cuticle or death by starvation [5]. Adult female boll weevils exposed to diflubenzuron lay eggs that do not hatch and mosquito larvae control can be achieved with as little as 1.0 gram of flucyclohexuron per acre of surface water [6], Su *et al.* [7] reported that Novaluron exhibited a high level of activity against *Culex* mosquitoes. Mulla *et al.* [8] showed that Novaluron exhibited long-term activity against *Aedes aegypti* in water-storage containers; it caused >80% larval mortality at 10 and 20 mg/kg of housefly. Many years after application of the IGRs, insects developed a resistance; [9] found resistance by *Musca domestica* toward Diflubenzuron and field

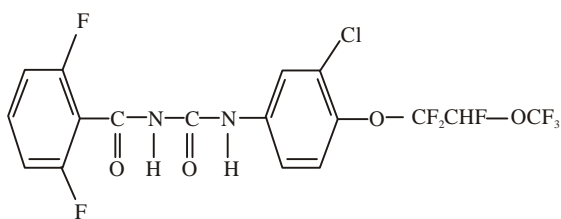


Fig. 1: Chemical structure of Novaluron [35]

Table 1: Some physicochemical properties of Novaluron [42]

Property	Value
Melting point	176.5-178.0°C
Water solubility	3 µg/l at 20°C, neutral pH
Log Octanol-water partition coefficient (log K_{ow})	4.3 at 20-25°C, pH 7.1
Vapor pressure	1.6 X 10 ⁻⁵ Pa at 25°C

populations with some resistance to cyromazine. As levels of insecticide resistance continue to increase, it is ever more important to develop alternative methods and insecticides for controlling the insects, but the toxicity toward ecosystem, non-target and beneficial organisms are increased. The present work investigates the contamination probability of freshwater microorganisms after treatment of water surfaces by pesticides to control mosquito, so we interested in the ecotoxicological activity of Novaluron (Rimon) on non-target organism which is *T. pyriformis* equipped with a chitinous exoskeleton membrane (like all ciliated microorganisms).

Novaluron N-[[[3-chloro-4[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]amino] carbonyl]-2,6-difluorobenzamide (Fig. 1), a relatively new CSI (Chitin synthesis inhibitor), that inhibits the chitin formation on larvae of various insects (Lepidoptera, Coleoptera, Homoptera and Diptera) [10]. It has a potent insecticidal activity against several important foliage feeding insect pests [11] and very low toxicity to mammals, birds and earthworms [12]. By inhibiting chitin formation, Novaluron selectively targets immature insect stages, causing abnormal endocuticular deposition abortive molting. While the incompatibility with natural enemies has been reported by Cutler *et al.* [13], the compound generally is selective in favor of non-target organisms [14, 15 and 16], giving it good potential in integrated pest management (IPM) programs. It had no effect on phytoseiid mite field populations [14], mortality and development of the soil-dwelling predatory mite, *Stratiolaelaps scimitus* (Womersley) [16] and greenhouse populations and percent parasitism of the parasitoid *Encarsia formosa* Gahan [15]. Novaluron with physicochemical properties shown in Table 1, is actually registered for usage against

L. decemlineata in the US (trade name Rimon) and is undergoing registration in Canada [13]. However, ecotoxicological effects of Novaluron on the ecosystem and especially on non-target microorganisms was not been evaluated. A little number of researches have studied the impact of pesticides on microorganisms [3, 4 and 17], but knowledge about ecotoxicological effects of Novaluron is till now ignored especially toward microorganisms beaded with structural chemical targets for this pesticide.

As treatment by pesticides is applied to the wide surfaces of soil and water, fish and other aquatic biota that were commonly used as bio-indicators of persistent organic pollutants [18] are usually exposed and organisms with structures that incorporate chemical target -in our case chitin- to pesticides are affected. Protozoa, algae and bacteria from broad base of food chains are often used as bioindicators of chemical pollution [19] especially in aqueous environment [20, 3, 4 and 21] because of the sensitivity of some organismal structures. Among protozoan, *T. pyriformis* is ubiquitous element of aquatic ecosystems. The short life cycle of 8h in axenic cultures allows easy cultivation under laboratory conditions. In addition, the cilia of *T. pyriformis* exhibit comparable characteristics with human respiratory epithelia-cells [22], so any impact on cilia could be extrapolated to human cells. This protozoa facilitates the study of biochemical and biological processes and effects on locomotory behavior by the microtubular system and mitochondria [23]. It was able to accumulate high amounts of Anthracene without any transformation [13]. In addition, it stores and secretes hormone-like materials known at higher level of phylogeny. These hormones are insulin, adrenocorticotrophic hormone, relaxin [24], endorphin [25]. As *T. pyriformis* is an aquatic organism, it may be affected by the insecticides treatment of water and soil, causing a perturbation in the aquatic chain food, especially when we put in mind that *T. pyriformis* has a chitinous membrane near in structure to this of insects [4], so we hypothesize that this protozoa could be a target of CSI, especially Novaluron.

MATERIALS AND METHODS

Cells Culturing and Treatments: *T. pyriformis* strain was used in the logarithmic phase of growth. The cells were grown at exponential phase in Proteose Peptone Yeast Medium (PPY), 2% proteose peptone and 5% yeast extract at pH 7.0-7.5, at 24±2°C. The density of *T. pyriformis* cultures was adjusted in fresh PPY in order to obtain at

least 10^4 cells per ml. Before the experiments on respiration metabolism, the cells were washed with fresh culture medium and were resuspended at the concentration of 5×10^4 cells ml^{-1} in 200ml flask; we take 1 ml to test in oxygraph (each time we added the appropriate concentration of novaluron to the reactive chamber by microsyringe). The cells were not exposed to Novaluron were used as control, the acetone is used to dissolve the pesticide so we obliged to investigate the impact of acetone on cells by addition of 5 $\mu\text{l/ml}$ to the medium cells (acetone-control). For the evaluation of Novaluron effect on *Tetrahymena* population growth, generation time and number, chitin integrity; we added the pesticide in culture medium before the addition of *Tetrahymena* cells, the used cells are starved for 96 h to become encysted. After the regeneration in culture medium that contains Novaluron, we investigate the effect on new chitin integrity.

Chemical Preparation: Novaluron is insoluble in water (3 $\mu\text{g/l}$ at 20°C) [26], so it is dissolved in acetone before adding to the distilled water in three concentrations 1, 10 and 20 μg of novaluron/5 μl acetone/1ml of culture medium. We are obliged to make an acetone control to eliminate the effect of solvent.

Growth Measurement: The growth of *T. pyriformis* population density is estimated by Lavergne [27] method (Optic density at $\lambda = 600$ nm), on aliquots of 2.5ml of cultures for each concentration with 3 repetitions, we used distilled water as white control, the cell number was determined by counting every cell present in 1 ml sample using a microscope and Petri box [28]. We have calculated the response percentage in 7th day, which evaluates the response of cells opposite the pollutant, described in Eq. 1 [29]:

$$RP = \frac{CN - EN}{CN} \times 100 \quad (1)$$

Where, *RP* is the Response percentage of protozoa (%); *CN* is the cell control number (cell/ml) and *EN* is treated cells number (cell/ml).

Determination of Generation Time and Number: Aliquot of 100 μl were immediately taken (T_0) from the control, control acetone and the exposed cultures and subsequently at 24 and 48 h (3 repetitions). the samples were diluted in distilled water and fixed with neutral-

buffered formalin (NBF) containing 10% (V/V) formalin in phosphate-buffered saline (PBS) (ph 7.4) at a final concentration of 2% to 5% for 1 h. the cell number was determined in each 30 μl under optical microscope. *T. pyriformis* cells were characterized by their generation time (*g*) required for doubling the population. Generation time and number were calculated using the following formulae [30].

Number of generation *n* is given by

$$n = \frac{\log N_1 - \log N_0}{\log 2} \quad (2)$$

Generation time *g* is given by:

$$g = \frac{\text{Time of growth}}{n} \quad (3)$$

where N_1 is the number of cells at 24 h, N_0 is the number of cells at T_0 and time of growth is 24 h.

Respiratory Metabolism: Protistes respiration was estimated using Clark's electrode (oxygraph), described by Djebbar and Djebbar [31], which go until nanomol of oxygen consumption. *T. pyriformis* at logarithmic phase (5×10^4 cells/ml) from homogenous culture was obtained and 1ml of culture is put inside reaction chamber of oxygraph, then we added the pesticide at appropriate concentrations 1, 10 or 20 μg , each trial was repeated 3 times, the respiration kinetic is followed for 20 minutes (after that time, cells became no stable) [31]. The results were registered directly as graphs on computer screen linked to the oxygraph.

Neutral red Technique: The effect of insecticide on chitin integrity was investigated using the method of Fournier [32], modified for protozoa by Rouabhi *et al.* [4]. Starved *T. pyriformis* is added to PPY + novaluron medium, after 96 h, the cells were appeared and we put a drop of neutral red with a drop of culture sample on microscope lame, after 3 minutes we took photos of control and exposed cells.

Statistical Study: All the experiments were repeated three times or more and the results were expressed as mean and standard deviation (SD) values. We used Minitab 15.1.1 software to make simple two-way ANOVA test with two criteria (treatment and time) and the test of Dunnett for comparison between the control and treated cells.

RESULTS AND DISCUSSION

The struggle against the agricultural ravagers was increased in few last years; many products were appeared and destined to kill the harmful insects, of which we did not know their effects on ecosystem and non-target animals. In the present work, we tried to investigate the possible ecotoxicological effects of Novaluron; an Insect growth Regulator (IGR) pesticide, on ecosystem and biomasses by the estimation of the growth, chitin and respiratory metabolism of *T. pyriformis* as a non-target aquatic organism exposed to this pesticide.

The impact of Novaluron on the population growth of *T. pyriformis* is shown in (Fig. 2). Indeed, Novaluron has an inhibitory effects on the population density growth of protiste in concentration-dependent manner, the highest concentration inhibits strongly the growth of *Tetrahymena* ($p < 0.001$). This result is confirmed by [3, 4] results on *Paramecium sp.* and *Tetrahymena pyriformis* treated with DFB, this due probably to the toxic effects on chitin synthesis by the Novaluron which incorporate on *N-glucosamine* [33], this result needs confirmation by the neutral red method, it is to note that Dunnett test shows a total difference between treated cells and the controls. The response percentage measurement results are presented in (Fig. 3). Gradual increase of 49.60 and 80% of response percentage, respectively of 1, 10 and 20 $\mu\text{g/ml}$. Microorganisms are highly sensitive to chemicals in aqueous environment. However, paramecia cells are relatively resistant to high concentration of acrylamide [34]. [35] found an increase in protein level of marine protozoa *Tetraselmis suecica* caused by the resistance phenomenon against aquatic pollutants, which confirm our results about response percentage. It is to note that acetone has not any toxic effect on protiste number and response ($p > 0.05$), Dunnett test revealed a difference between treated cells and control.

Under the culture conditions used in this study, the initial cell density was 1.5×10^4 cells/ml and the normal generation time of *Tetrahymena pyriformis* was about 7.2 h. the experiments showed that the addition of Novaluron affects gradually generation time (Table 2) in dose-dependent manner. The acetone and Novaluron in lower concentration (1 $\mu\text{g/ml}$) did not have a significant effect ($p > 0.01$) on generation time of *Tetrahymena pyriformis*. However, generation time was increased at higher concentrations (10 and 20 $\mu\text{g/ml}$) of Novaluron, indicating that Novaluron of higher concentration inhibits the growth of *Tetrahymena pyriformis* ($p < 0.001$), Dunnett test showed a difference of the cells treated by

Table 2: Effect of Novaluron on generation time of *T. pyriformis* after 24 h of growth. Each value is means \pm SD of three independent observations

Samples	Generation time/h
Control	7.24 \pm 0.04
Acetone-control	7.27 \pm 0.35
Novaluron (1 $\mu\text{g/ml}$)	7.59 \pm 0.61*
Novaluron (10 $\mu\text{g/ml}$)	8.71 \pm 1.03***
Novaluron (20 $\mu\text{g/ml}$)	9.50 \pm 1.52***

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

10 and 20 $\mu\text{g/ml}$ to the control. [3, 4] reported that the Benzoylphenyl ureas inhibit the growth of protozoa by the reduction of chitin thickness, also this reduction it may be due by the incorporation of Benzoylphenyl ureas in RNA of protozoa [36]. [37] reported that Toxics may affect the survival of protista in a variety of ways, as the concentration of toxicants in the cell membranes and destroy their integrity causing lysis, or by effect on enzymes, inactivating them by binding to sulphhydryl, amino and amino groups of enzyme protein.

To confirm the effect of Novaluron on growth the investigation on chitin was established, the neutral red coloration method was used and the results are shown in (Fig. 5). The treatment with Novaluron induce a massive penetration of neutral red especially at the highest concentrations where the red color is focused in digestive vacuoles and on the borders, this due to the porosity of the membrane, which is altered by the Novaluron. Soltani *et al.* [38] have showed that the DFB and the FCX affected the development of *Tenebrio molitor* by the inhibition of chitin synthesis, also Cutler *et al.* [39] have showed that novaluron acting on chitin of Colorado potato beetle. The swim and the form of cells were affected, also we noted a big number of rounded cells (encysted form), which translates the toxic effect of Novaluron on *T. pyriformis*. The swim is affected probably by the effect of the pesticide on the mitochondria (ATP), which induce apoptosis phenomenon translated by the increased number of encysted cells (dead cells) [35], the impact on mitochondria confirmed also by the swimming perturbation because the movement of lashes needs the consummation of ATP produced from mitochondria.

Effect of Novaluron on the respiratory metabolism of *T. pyriformis* was investigated for 20 minutes (acute toxicity) using Hansatech electrode described by [31], the results are illustrated in (Fig. 4). Indeed, 20 $\mu\text{g/ml}$ of novaluron inhibits *T. pyriformis* respiration ($p < 0.001$), dunnett test revealed that cells treated with 1 $\mu\text{g/ml}$ of Novaluron is not different to control. Wherever, the

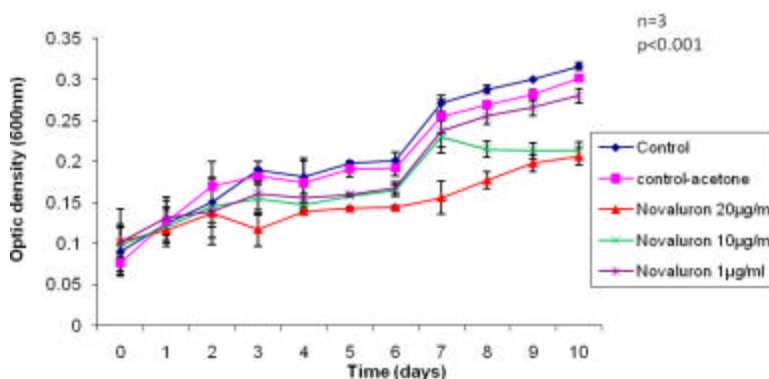


Fig. 2: Impact of Novaluron on growth of *T. pyriformis* population number according the time ($p<0.001$). Each value is means \pm SD of three independent observations

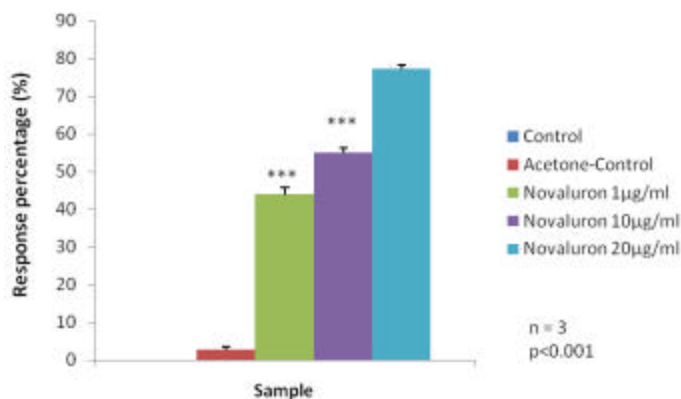


Fig. 3. Response percentage of *T. pyriformis* in presence of 1, 10 and 20 μ g/ml of novaluron ($p<0.001$) at 7th day. Each value is means \pm SD of three independent observations

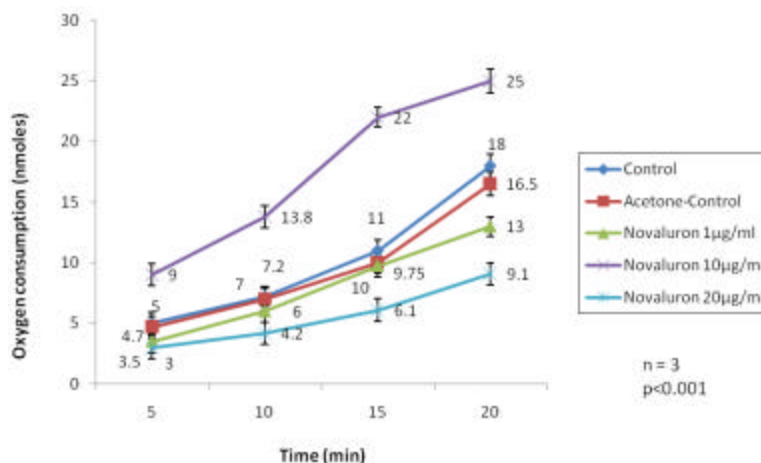


Fig. 4: Impact of Novaluron in three concentrations (1, 10 and 20 μ g/ml) on respiratory metabolism of *T. pyriformis*. Each value is means \pm SD of three independent observations

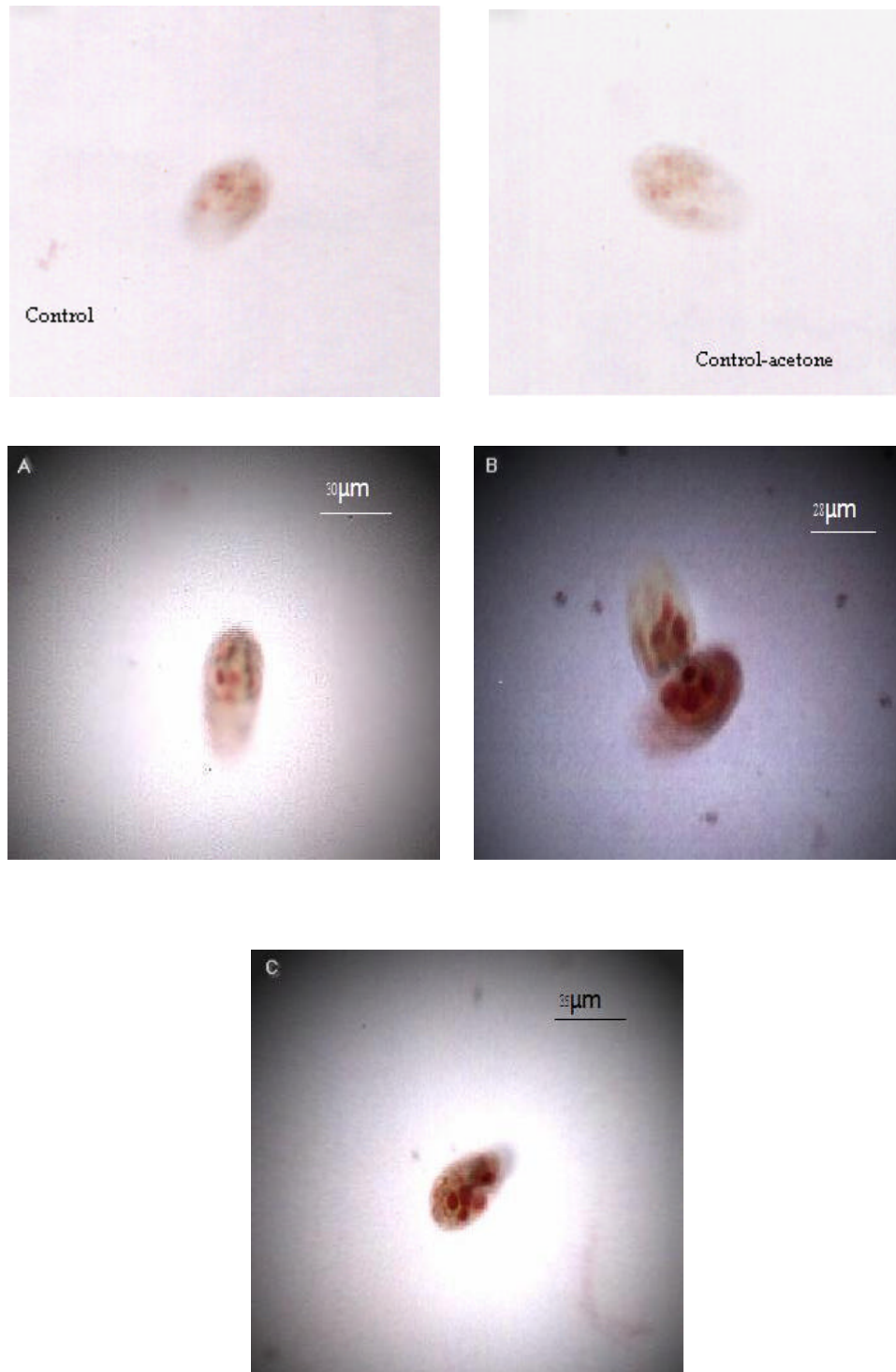


Fig. 5: Impact of 1, 10 and 20 μ g/ml of Novaluron on chitin integrity of *Tetrahymena pyriformis*, A: treated with 1 μ g/ml; B: treated with 10 μ g/ml; C: treated with 20 μ g/ml (80X)

treatment by 10 µg/ml increases the oxygen consumption ($p < 0.001$) according to the control (from 5 to 18 nmol). This result is explained if we base on the detoxification/metabolisation mechanisms by mono-oxygenases enzymes, where the cells consummate O₂ to make the substrate more hydrophilic so eliminated by water. These enzymes are coupled with substrate in the cells treated with 10 µg/ml of novaluron, but saturated or blocked in cells treated with the two other concentrations (1 and 20 µg/ml) [4]. The decrease of oxygen consumption in the highest concentration of Novaluron is also a signification of the reduced number of cells because we started from the same number of cells.

In conclusion, our study showed that the highest concentrations of Novaluron, C₁₇H₃ClF₈N₂O₄, caused a dose-dependent growth inhibition of *T. pyriformis* population. The results suggest that the antiproliferative effect of Novaluron may be mediated by reducing the chitin and cuticle of cells, also by the effect on mitochondria and lashes, so Novaluron has toxic effects on *T. pyriformis* by reduction of growth, increasing of generation time, perturbation of respiratory metabolism, high response percentage and alteration of chitin rigidity and integrity. All results showed that this pesticide has an ecotoxicological effects on non-target organisms and ecosystem. It is to note that the used concentrations are high compared with concentrations found to effect aquatic crustaceans and insects, this due to the response and the physiology of *T. pyriformis* cells which are comparable to human and high organisms.

These results fill the gap concerning the effect of chitin synthesis inhibitors on non-target organisms (protozoa) that involve chitin in their structure.

REFERENCES

1. Turco, R.F., A.C. Kennedy and M.D. Jawson, 1994. Microbial indicators of soil quality. In: Defining soil quality for sustainable environment, Oran, J.W., D.C. Coleman, D.F. Bezdicek and B.A. Stewart (Eds.), Special Pub. 35. Soil Science Society of America, Madison, WI, pp: 73-90.
2. Soltani, N., S. Chebira, N. Pitoizet, J.P. Delbecoque and J. Delachambre, 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.
3. Rouabhi, R., H. Djebbar and M.R. Djebbar, 2006a. Toxicity evaluation of flucycloxuron and diflubenzuron on the cellular model, *Paramecium sp.* African J. Biotechnol., 5(1): 045-048.
4. Rouabhi, R., H. Djebbar-Berrebah and M.R. Djebbar, 2006b. Toxic Effect of a Pesticide, Diflubenzuron on Freshwater Microinvertebrate (*Tetrahymena pyriformis*). Chinese J. Applied Environ. Biol., 12(4): 514-517.
5. Chebira, S., N. Soltani-Mazouni and N. Soltani, 2000. Evaluation of two insect growth regulators, Flucycloxuron and Diflubenzuron, affecting *in vivo* and *in vitro* the ecdysteroid production in *Tenebrio molitor* (Coleoptera), In Proceedings, 10th Intl. Conference of Cairo University, pp: 75-89.
6. Ware, G.W., 2000. The Pesticide Book, 5th Edn. Thomson Publications, Fresno, California.
7. Su, T.Y., M. Zaim and M.S. Mulla, 2003. Laboratory and field evaluation of novaluron, a new insect growth regulator (IGR) against *Culex* mosquitoes. J. American Mosquito Control Assoc., 19: 408-418.
8. Mulla, M.S., U. Thavara, A. Tawatsin, J. Chomposri, M. Zaim and T. Su, 2003. Laboratory and field evaluation of novaluron, a new acylurea insect growth regulator, against *Aedes aegypti* (Diptera: Culicidae). J. Vector Ecol., 28: 241-254.
9. Kristensen, M. and J.B. Jespersen 2003. Larvicide resistance in *Musca domestica* (Diptera: Muscidae) populations in Denmark and establishment of resistant laboratory strains. J. Econom. Entomol., 96: 1300-1306.
10. Tomlin, C.D.S., 1997. Novaluron. In: The pesticide manual, Tomlin, C.D.S. (Ed.). 11st Edn. British Crop Protection Council, UK, pp: 888-889.
11. Cutler, G.C., C.D. Scott-Dupree, J.H. Tolman and C.R. Harris, 2005a. Acute and sublethal toxicity of Novaluron, a novel chitin synthesis inhibitor, to *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). J. Pest Manage. Sci., 61:1060-1068.
12. Ishaaya, I. and AR. Horowitz, 1998. Insecticides with novel modes of action: An overview. In: Insecticides with novel modes of action, Ishaaya, I. and D. Degheele (Eds.). Springer, Berlin, Germany, pp: 1-24.
13. Cutler, G.C., C.D. Scott-Dupree, J.H. Tolman and C.R. Harris, 2006. Toxicity of Novaluron to the non-target predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae). Biological Control, 38: 196-204.

14. Guiraud, P., J.L. Bonnet, A. Boumendjel, M. Kadri-Dakir, M. Dusser, J. Bohatier and R. Steiman, 2008. Involvement of *Tetrahymena pyriformis* and selected fungi in the elimination of anthracene and toxicity assessment of the biotransformation products. *Ecotoxicol. Environ. Safety*, 69: 296-305.
15. Ishaaya, I., S. Kontsedalov, D. Mazirov and A.R. Horowitz, 2001. Biorational agents: Mechanisms and importance in IPM and IRM programs for controlling agricultural pests. *Medical Faculty of Landbouww. Univ. Gent.*, 66: 363-374.
16. Ishaaya, I., A.R. Horowitz, L. Tirry and A. Barazani, 2002. Novaluron (Rimon) a novel IGR: mechanism, selectivity and importance in IPM programs. *Medical Faculty of landbouww. Univ. Gent.*, 67: 617-626.
17. Cabera, A.R., R.A. Cloyd and E.R. Zaborski, 2005. Lethal and sublethal effects of Novaluron (Pedestal) on the soil-dwelling predatory mite, *Stratiolaelaps scimitus* (Womersley) (Acari: Mesostigmata: Laelapidae), under laboratory conditions. *J. Entomol. Sci.*, 40: 47-53.
18. Van der Oost, R., J. Beyer and N.P.E. Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A Rev. *Environ. Toxicol. Pharmacol.*, 13: 57-149.
19. Nalecz-Jawecki, G., K. Demkowicz-Dobrzahski and J. Sawicki, 1993. Protozoan *Spirostomum ambiguum* as a highly sensitive bioindicator for rapid and easy determination of water quality. *The Science of the Total Environment, Supplement*, pp: 1227-1234.
20. Foissner, W., 1999. Soil protozoa as bioindicators: Pros and cons, methods, diversity, representative examples. *Agric., Ecosyst. Environ.*, 74: 95-112.
21. Mohd, M.H., R.A. Nageswara, R.S. Venkata and B. Mohan, 2007. Low cost micro bioassay test for assessing cytopathological and physiological responses of ciliate model *Paramecium caudatum* to carbofuran pesticide. *Pesticide Biochemistry and Physiology*. DOI: 10.1016/j.pestbp.2007.07.006.
22. Krejè, S., O. Herbarth, S. Rudzok, E. Schmücking and A. Müller, 2007. Chronic effects of metals, organophosphates and polycyclic aromatic hydrocarbon (PAH) on *Tetrahymena pyriformis*. *Abstracts/Toxicology Letters*, 172S: S84-S85.
23. Kovács, P., G. Csaba, E. Pallinger and R. Czaker, 2007. Effects of taxol treatment on the microtubular system and mitochondria of *Tetrahymena*. *Cell Biol. Intl.*, pp: 724-732.
24. LeRoith, D., J. Schiloach, M. Berelowitz, L.A. Forhmann, A.S. Liotta, B.T. Krieger and J. Roth, 1983. Are messenger molecules in microbes the ancestors of the vertebrate hormones and tissue factors? *Fed process*, 42: 2602-2607.
25. Csaba, G. and P. Kovács, 1999. Localization of δ -endorphin in *Tetrahymena* by confocal microscopy. Induction of the prolonged production of the hormone by hormonal imprinting. *Cell Biol. Intl.*, 23: 695-702.
26. WHO., 2004. Novaluron. (\pm)-1-[3-chloro-4-(1,1,2-trifluoro-2-rifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea. Geneva, World Health Organization (WHO Specifications and Evaluations for Public Health Pesticides.
27. Lavergne, M., 1985. Étude des effets du Diazépam et du Medazepam sur un protiste cilié. Mémoire de DEA, de l'Université de Paris VII.
28. Gerson, M. and D. Stainier, 1995. Culturing *Tetrahymena* as an alternative baby food to paramecia. TP. Report, Department of Biochemistry and Biophysics, UCSF School of Medicine, San Francisco, CA.
29. Wong, D.C.L., E.Y. Chai, K.K. Chiu and P.B. Dorn, 1999. Prediction of ecotoxicity of hydrocarbon-contaminated soils using physico-chemical parameters. *Environ. Toxicol. Chem.*, 18: 2611-2621.
30. Dias, N., R.A. Mortara and N. Lima, 2003. Morphological and physiological changes in *Tetrahymena pyriformis* for the *in vitro* cytotoxicity assessment of Triton X-100. *Toxicology in vitro*, 17: 357-366.
31. Djebar, M.R. and H. Djebar, 2000. Bioénergétique, les mitochondries végétales. *Revue des Sciences et Technologies, Synthèse, Publication de l'Université d'Annaba*. Vegator (Eds).
32. Fournier, E., 1993. *Toxicologie*. Edition Ellipses.
33. Nakagawa, Y., F. Matsunura and Y. Hashino, 1993. Effect of DFB on incorporation of [H^3]-N-acetyl glucosamine ([H^3] NAGA) into chitin in the intact integument from the newly molted American cockroach *Periplaneta Americana*. *Comp. Biochemistry and Physiology*, 106C : 711-715.
34. Takahashi, T., M. Yoshii, T. Kawano, T. Kosaka and H. Hosoya, 2005. A new approach for the assessment of acrylamide toxicity using a green paramecium. *Toxicology in vitro*, 19: 99-105.

35. Rouabhi, R., 2007. Impact de deux pesticides le Diflubenzuron et le Flucycloxyuron sur trois modèles cellulaires alternatives: *Paramecium* sp., *Tetrahymena pyriformis*, *Tetraselmis suecica* et sur le développement embryonnaire de la poule domestique (*Gallus domesticus*). Thèse de Doctorat de l'université d'Annaba, Option: Toxicologie appliquée, Université de Annaba, Algérie.
36. Klitschka, G.E., R.T. Mayer, R.E. Droleskey, J.O. Norman and A.C. Chen, 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.
37. Madoni, P., 2000. The acute toxicity of nickel to freshwater ciliates. *Environ. Pollution*, 109: 53-59.
38. Soltani, N., S. Chebira, J. Delbecque and J. Delachambre, 1993. Biological activity of Flucycloxyuron, a novel benzoylphenylurea derivate, on *Tenebrio molitor*: comparison with Diflubenzuron and Tiflumuron. *Experientia*, 49(12): 1088-1901.
39. Cutler, G.C., C.D. Scott-Dupree, J.H. Tolman and C.R. Harris, 2007. Field efficacy of Novaluron for control of Colorado potato beetle (Coleoptera: Chrysomelidae) on potato. *Crop Protect.*, 26: 760-767.
40. Tayeb, I. and L. Mansour, 2007. Étude du comportement de *Tetrahymena pyriformis* à la présence d'un inhibiteur de croissance, le Novaluron. Mémoire d'ingénieur d'état en physiologie animale, centre universitaire de Tébessa, Algérie.
41. WHO., 2007. Novaluron in Drinking-water: Use for Vector Control in Drinking-water Sources and Containers. Background document for development of WHO Guidelines for Drinking-water Quality.