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Behavior of *Paramecium* Sp., Treated with Bifenazate with Special Emphasis on Respiratory Metabolism, Protein and Generation Time

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Abstract: The human species has never been as vulnerable to its environment, particularly in relation to the disturbances that it even introduced. The problem is essentially that of the urban environment soon where 90% of the population lives, while the sources of toxic exposure to electromagnetic fields continue to multiply. Currently in toxicology, the use of alternative models permits to understand the mechanisms of toxic action at different levels of the organization of the cell. The main objective of our work is to study the effect of the acaricide: the Bifenazate on ciliate protists freshwater: *Paramecium sp.* To do this, we tested the effect of this xenobiotic at different concentrations (10, 20, 40 and 80µM) on aliquots of 50ml of culture of paramecium done beforehand. The results obtained show that the growth of paramecium is a sensitive to the product and at the high concentrations. The toxicity was evaluated by determining the IC 50 and by calculating the percentage response, which evaluates the response of protists towards the pollutant and thus confirms the evolution of the growth curve. The variation in the rate of total protein concentrations according to Bifenazate compared to the control reflects the ability of microorganisms to the metabolisation/ detoxification. The measurement of respiratory activity shows inhibition of oxygen consumption reflecting a fatal effect of this acaricide.

Key words: Pollution · Acaricide · Bifenazate · Tests cytotoxicity · IC50 · Protein · Respiratory metabolism

INTRODUCTION

Pesticides are chemical products used to eliminate the harmful organisms, but most of them will also affect the non-target or beneficial organisms, with direct way (absorption, ingestion, respiration, etc...) or indirect way (via another contaminated, polluted water, etc.). The effects on biodiversity, including flora and fauna in terrestrial and aquatic, are undeniable.

Several phenomena may, in fact, increase the impact of pesticides on wildlife. Between these: the spread of products, non-selectivity and toxicity of the active molecule, the persistence of the molecule and its ability to accumulate in the food chain and finally the form and preparation of pesticide.

Over 90% of synthetic insecticides are organophosphates, carbamates and pyrethroids with action on the nervous system [1]. Among these products we have chosen an insecticide the Bifenazate.

For the first, discovered in 1990 by Uniroyal Chemical and marketed by Crompton Corporation in 1999 [2-4], it is a selective acaricide of the family of Hydrazine carboxylate which can control a variety of mite pests of greenhouse ornamentals, apples, pears, citrus fruits and cotton. Preliminary studies on its mode of action indicate that the high concentrations of Bifenazate act on receptors on post-synaptic GABA nervous system of insects [5, 4]. However, this information has not yet been supported by researches; the Bifenazate is classified in group 25 from the IRAC (Insecticide Resistance Action Committee) inhibitor neurons, but with unknown mode of action.

This study was undertaken to better characterize the mechanism of action of this pesticide at different levels of organization of the cell. They allow to specify the interactions between a test molecule and target cells and to identify the disturbed metabolism, altered or induced by these elements to explain either their toxicity or their tolerance by the cell.

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MATERIALS AND METHODS

Chemicals: The used acaricide in our work was provided by Professor Guy Smagghe laboratory Director of Agrozoology in Gent University, Belgium (Fig 1).

Cell Culture and Treatment: The culture medium of paramecium was performed according to the method of [1].

Xenobiotic was tested in aliquots of 50ml of culture, four concentrations were chosen: 10, 20, 40 and 80µM (the tests are repeated three times and results are expressed as the mean ± Error.

Kinetics of Growth: The growth kinetics of paramecium is done by measuring the optical density (OD) at wave length λ = 600 nm [6].

Calculating the Percentage Response and the IC_{50}: The toxicity was evaluated by determining the IC_{50}, which determines the concentration which, under standard conditions, inhibits 50% of population [7] and the percentage of response which assesses response of protists in presence of the pollutant, according to the equation:

$$PR = [(NC - BN) / NC] \times 100 \quad [1]$$

The positive values of response percentage indicate an inhibition of growth, while negative values indicate a stimulation of growth.

Determination of Total Protein: Proteins are determined by the method of [8]. The density is measured at a wavelength of 595nm by spectrophotometer type JENWAY 3600.

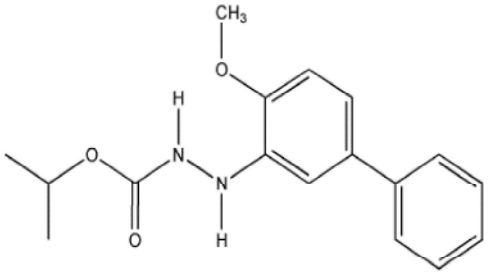

Fig. 1: Bifenazate Chemical Formula

Polarographic Measurement: The used machine is an oxygen electrode, type HANSATECH, which allows the measurement of oxygen output or consumption [9], in the order of nanomole.

Statistical Analysis: The analysis of variance with two controlled factors is used to estimate the differences reported for the different studied parameters.

RESULTS

Effect of Bifenazate at Different Concentrations: Figure (2) shows a decrease in the growth of cells up to the 4th day, for grow thereafter and achieve the 6th day OD = 0.180 nm. In parallel, cells treated with 10 and 20µM illustrate a condition almost identical to that of Controls. For those treated with 40 and 80µM, a gradual inhibition of cell growth is observed almost from the 1st day of treatment

Calculating the Response Percentage and the IC_{50}: The percentage of response [1] which is a parameter for evaluating the effect of the pesticide at different concentrations confirms the results obtained concerning the kinetics of growth of the studied microorganism.

Figure (3) shows that the response percentage of paramecium is dose-dependent and proportional to increasing concentrations of the Bifenazate, it is 5.55% for the concentration of 10µM to thereafter 62.77 % at 80µM where over half the population is inhibited by this dose.

To characterize the toxicity, we determine the 50% inhibitory concentration (IC_{50}). The rates corrected of normality obtained are transformed into probite and allow establish a straight line regression based on the decimal logarithms of the doses used. According the curve, we can determine all the outstanding doses (IC_{50}, IC_{16}, IC_{84}) and the Slope (the slope of the regression line).

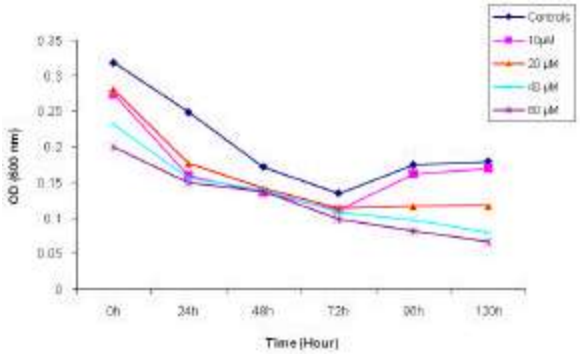

Fig. 2: Effect of Bifenazate on paramecium cell growth according the time in Hours (P<0.001)

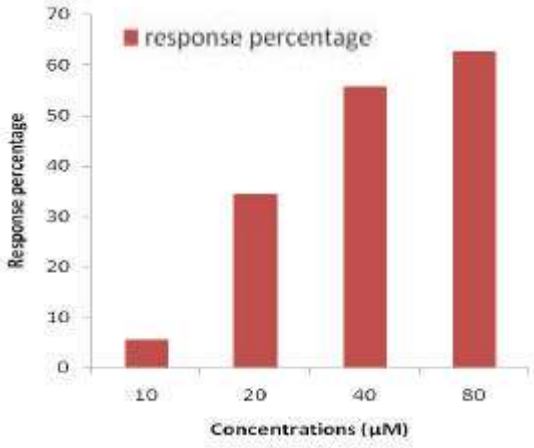

Fig. 3: Evolution of the response percentage of paramecium in presence of different concentrations of Bifenazate

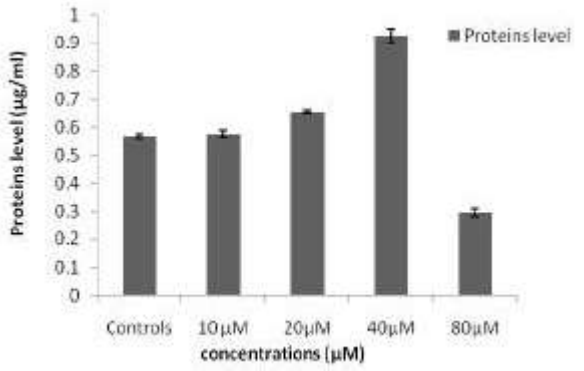

Fig. 4: Variation in the rate of total protein level according to concentrations of Bifenazate, $p<0.001$

Table 1: Determination of IC_{50}

	Bifenazate	
Exposition	IC_{50} (µM)	Slope
72 h	261.8	0.058
96 h	57.22	0.295
120 h	40.90	0.477

Note that the inhibitory concentration decreases with the duration of exposure (Table1).

Effects of Bifenazate on the Rate of Total Protein: Changes in the rate of total proteins obtained are represented in Figure4.

We note that the protein rate increases according to concentrations of Bifenazate compared to the control, it decreased thereafter to 80µM.

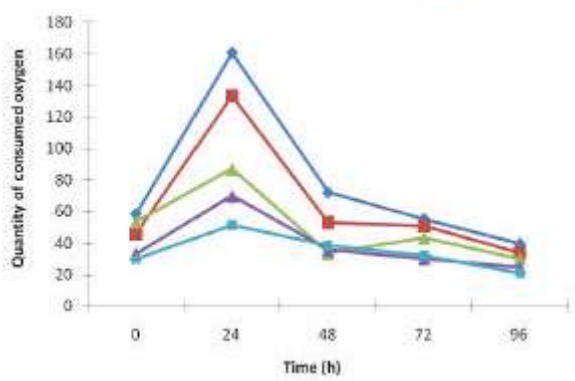

Fig. 5: Effect of Bifenazate on the respiratory metabolism of paramecium

Effects of Bifenazate on the Respiratory Metabolism: Figure (5) illustrates the effect of product on the respiratory metabolism during 5 days (96 h) of treatment. At low concentrations (10 and 20 µM) the amount of consumed O_2 decreases slightly compared to the control. In high concentrations (40 and 80µM) the amount of consumed O_2 decreases significantly compared to controls (and even at 24 hours)

DISCUSSION

Water quality is essential for the maintaining the health of aquatic organisms, the presence of toxic substances in water is a threat to organisms that living there. The report of the agency for protection of the environment of 1990 suggests that the pollution of rivers and ponds come mainly from agriculture [10]. Many pesticides are used indiscriminately by the farmers to control pests. They cause water pollution and affect the organisms including protozoa, especially the paramecium [11].

The paramecium cells were used in the past for the rapid assessment of the toxicity of pesticides [12-15]. These protists, ubiquitous in aquatic and terrestrial environment, characterized by a short life cycle [16] which led us to use them as cellular models to study the impact of xenobiotics and the assessment of health risks [6].

The Evaluation of cytotoxic effects of a xenobiotic can be performed using different parameters, including cell growth, which reflects the state of cell metabolism [6,17].

Thus our results show that at low concentrations, the Bifenazate has little effect on cell growth, this could be either:

- The adsorption of xenobiotics on the cell membrane and the presence of cuticle in paramecium, which make them resistant but remain nevertheless permeable [18].
- To reach their molecular targets, the acaricides penetrate inside the body through either the cuticle or the walls of the digestive tract. This penetration occurs at a speed which, for the same toxic, varies from one species to another. If the kinetics of penetration is sufficiently slow, the acaricide can be degraded by the detoxification systems and will have little effect [19].

The percentage of response [1] confirms the trend of growth curves of paramecium processed, thus it seems clear that the xenobiotic is inhibitors of the growth of paramecium in high concentrations.. In fact, the growth highlights a slight inhibition to approximately 72 hours and for low concentrations with an IC_{50} of 261.8µM. These results are consistent with those of [11] which showed a decrease in population density of paramecium exposed to 1 and 100 ppm of Monocrotophos (MCP). The same is true for [1, 14]. Our results are the same lines as those of [20] that worked on *Phytoseiulus persimilis*, *Amblyseius fallacis* and nymphs of *Orius insidiosus* where the LC_{50} of *A. fallacis* is 7.6 times higher than what is indicated on the packaging.

However, inefficiency of Bifenazate at low concentrations is because it is one of the few acaricides which contains no heavy metals or halogens which could cause different toxic effects when applied at high doses [21].

The Bifenazate is a selective acaricide reported as Neurotoxic but this information has not yet been supported by studies. It is always regarded as an inhibitor of neurons, but we do not know the exact mode of action [4, 5]. According to [21], they suggested that the Bifenazate acts on another site that the receptors post-synaptic GABA, most likely encoded by the mitochondrial genome.

The increased dose-dependent of total protein rate could be explained by the fact that the presence of xenobiotics in the cell stimulates protein synthesis of many enzymes and reflects the ability of these microorganisms to the metabolisation/detoxification as with the enzyme Cyt P_{450} Monooxygenases [22] or by conjugation reactions [19].

Our results are consistent with this work and confirm the role of stress proteins in microorganisms [23]. It is obvious that the rate of total protein alone does not provide a clear explanation of the mechanisms for detoxification/biotransformation by the paramecium in the presence of xenobiotics, so we have oriented our work towards finding a possible disturbance of the respiratory metabolism.

Our results showed a perturbation of the respiratory metabolism of microorganisms treated with different concentrations of the studied xenobiotic compared to control cells. Our results are consistent with those of [24] who tested the effect of gossypol on the morphology, mobility and metabolism of *Dunaliella bioculata* (flagellate protists) regarded as a cell model of human sperm.

It seems clear that oxygen is an essential element for live of microorganisms and at the time of elimination of xenobiotics, the electrons produced at the detoxification of the body by Cyt P_{450}, will react with the oxygen. Oxygen can also react with the electrons that escape from the respiratory chain [1]. The direct combination of oxygen with these electrons involves the formation of a superoxide anion which is the origin of the radical phenomena.

The perturbation of the respiratory activity obtained in our work shows that low concentrations of Bifenazate generates an oxidative stress that led to release of ROS which are known as disruptive of respiratory metabolism [25, 26]. The major role of the endogenous production of ROS is the regulation of the activity. Indeed, the radicals can interact directly with molecules containing sulfhydryl groups and therefore change their conformation. This type of regulation may affect the particular molecules involved in transduction mechanisms, such as protein-kinase C [27].

These ROS are quickly neutralized by the system of defense/detoxification using O_2 molecules for their activity; it is so monooxygenases and oxidases. The high respiratory activity recorded at 24 hours supports this finding. Also after 24h and after trigger of the defense system of the paramecium, the changes initially recorded at 24 hours are reduced strongly beyond this time.

Parallel, high concentrations of Bifenazate causes a sharp reduction in respiratory activity of cells closely related to the decrease in the number of paramecium, with a strong release of ROS capable of interfering with the of the respiratory chain components, causing malfunction, or even blockage, this leads to the apoptosis [1].

After considering all the experimental data obtained throughout the study, it appears that the ciliate protists used in our work is a material of choice for studies in toxicology and occupies a privileged position in aquatic

ecosystems because it is one of the basic elements of food chain, hence the need for a deep study of the impact of pollution on our environment.

REFERENCES

1. Rouabhi, R., H. Djebar-Berrebbah and M.R. Djebar, 2008. Growth, Chitin and respiratory metabolism of *Tetrahymena pyriformis* Exposed to the insecticide Novaluron. Am-Euras. J. Agric. and Environ. Sci., 3(6): 873-881.
2. Dekeyser, M.A. and P.T. McDonald, 1994. Insecticidal phenyl hydrazine derivatives. US Patent US 5367093.
3. Dekeyser, M.A., P.T. McDonald, J.R. Angle and R.C. Moore, 1996. D2341 a novel agent to control spider mites. In Proceeding of the Brighton Crop Protection Conference, Pests and Diseases, BCPC, Farnham, Surrey, UK. pp: 487-492.
4. Grosscurt, A.C. and L. Avella, 2005. Bifenazate, a new acaricide for use on ornamentals in Europe and Africa. Proceeding of the BCPC International Congress, Crop Science and Technology, pp: 49-56.
5. Dekeyser, M., 2005. Acaricide mode of action. Pest Manage. Sci., 61:103-110.
6. Sauvant M.P., D. Pepin and E. Piccini, 1999. *Tetrahymena pyriformis*: A tool for toxicological studies. Chemosphere, 38(7): 1631-1669.
7. Bonne,t J.L., P. Guiraud, M. Dusser, M. Kadri, J. Lafosse, R. Steiman and J. Bohatier, 2003. Assessment of anthracene toxicity toward environmental eukaryotic microorganisms: *Tetrahymena pyriformis* and selected micromycetes. Ecotoxicology and Environmental Safety, 60: 87-100.
8. Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.
9. Djebar, M.R., 1989. Compte rendu de l'académie des sciences. Paris III
10. Cook, J.L., P. Bauman, J.A. Jackman and D. Stevenson, 1995. Pesticide Characteristics that affect water quality. Farm. Chemical Handbook, Meister Publishing Co. Willoughby. pp: 429.
11. Ujwala, G., N. Shanti Desai and V. Prakash Desai, 2007. Toxic effects of Monocrotophos on *Paramecium caudatum*. Afr. J. Biotechnol., 6(19): 2245-2250.
12. Komala, Z., 1992. Toxicity of Fastac 10EC, a pyrethroids insecticide to *Paramecium primaurelia* and *Tubifex sp*. Folia Biol. (Krakow). 40(3): 109-112.
13. Juchelka, C.M. and T.W. Snell, 1995. Rapid toxicity assessment using ingestion rate of Cladocerans and Ciliates. Arch. Environ. Contam. Toxicol., 28: 508-512.
14. Rouabhi, R., H. Berrebbah and M.R. Djebar, 2006. Toxicity evaluation of flucycloxuron and diflubenzuron on the cellular model, *Paramecium sp*. Afr. J. Biotechnol., 5(1): 45-48.
15. Rao, V.J., V.G. Gunda, K. Srikanth and S.K. Arepalli, 2007. Acute toxicity bioassay using *Paramecium caudatum*, a key member to study the effects of monocrotophos on swimming behavior, morphology and reproduction. Toxicol. Environ., 89(1): 1080-1086.
16. Beal, D.L. and R.V. Anderson, 1993. Response of Zooplankton to rotenone in a small pond. Bull. Environ. Contam. Toxicol., 51: 551-556.
17. Perez-Rama, M., A.J. Abalde, L.C. Herrero and E. Torres, 2001. Class III Metallothioneins in Response to Cadmium Toxicity in The Marine Microalga *Tetraselmis suecica* (Kylin) Butch. Environmental Toxicology and Chemistry, 20(9): 2061-2066.
18. Beaumont, R. and C. Cassier, 1998. Travaux Pratiques de Biologie Animale, Zoologie, Embryologie, Histologie, 3ème édition. Dunod Ed., pp: 123-143.
19. Haubruge, E. and A. Marcel, 1998. Les mécanismes responsables de la résistance aux insecticides chez les insectes et les acariens. Biotechnol. Agron. Soc. Environ., 2(3): 161-174.
20. Bostanian, N.J. and M. Akalach, 2006. The effect of indoxacarb and five other insecticides on *Phytoseiulus persimilis* (Acari: Phytoseiidae), *Amblyseius fallacis* (Acari: Phytoseiidae) and nymphs of *Orius insidiosus* (Hemiptera: Anthocoridae). Pest Manag. Sci., 62(4): 334-339.
21. Thomas, V.L., T. Luc and N. Ralf, 2006. Complete maternal inheritance of Bifenazate resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae) and its implications in mode of action considerations. Insect Biochemistry and Molecular Biology, 36: 869-877.
22. Anzenbacher, P., 2001. Cytochromes P_{450} and metabolism of xenobiotics Review CMLS. Cell. Mol. Life Sci., 58: 737-747.
23. Masaya, M., H. Yoshinobu, Y. Ai, K. Maki and O. Yasuo, 2002. Determination of cellular levels of non protein Thiol in phytoplankton and their correlations with suseptibility to mercury. Journal of Phycology, 38(5): 979-983.

24. Druez, D., F. Marano, R. Calvayrac, B. Volochine and J.C. Soufir, 1989. Effect of gossypel on the morphology, mobility and metabolism of a flagellated protist, *Dunaliella bioculata*. J. Submicrosc. Cytol. Pathol., 21(2): 367-374.
25. Kiss, S.A., I.S. Varga, Z. Galbacs, T.H. Maria and A. Csikkel-Szolnoki, 2003. Effect of age and magnesium supply on the free radical and antioxidant content of plants. Acta Biologica Szegediensis, 47(1-4): 127-130.
26. Kuciel, R. and A. Mazurkiewicz, 2004. Formation and Detoxification of reactive oxygen species.Biochemestry and Molecular Biology Education, 323(17): 183-186.
27. Dalton, T.P., H.G. Shertzer and A. Puga, 1999. Regulation of gene expression by reactive oxygen. Ann. Rev. Pharmacol. Toxicol., 39: 67-101.