### ABSTRACT

The toxicological impacts of the increasing number of synthetic compounds present in the aquatic environment are assessed predominantly in laboratory studies where test organisms are exposed to a range of concentrations of single compounds. The bioindicator *Paramecium sp.*, characterized by a short life cycle, rapid multiplication and normal behavior that may be affected by the presence of pollutants. We therefore investigated the inhibitory effect of a newly synthesized acaricide: the chlorfenapyr tested at concentrations of 250, 300 and 350 µM on a pure culture of *Paramecium sp.* during 6 day. Paramecia treated with different concentrations of Chlorfenapyr illustrate strong inhibition of cell growth from the second day of treatment. Low levels of glutathione, increased glutathione S-transferase and the decrease in respiratory metabolism, recorded in the presence of different concentrations of Chlorfenapyr, involve the activation of detoxification system.

**Keywords:** Chlorfenapyr, detoxification oxidative enzymes, *Paramecium sp.*

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**INTRODUCTION**

The extensive use of insecticides, during the past decades has led to a number of negative effects on terrestrial and aquatic organisms. Chlorfenapyr is a member of a new class of chemicals, namely, pyrroles (chemical name: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrole-3-carbonitrile; trade name: Pylon miticide-insecticide). Chlorfenapyr uncouples oxidative phosphorylation in the mitochondria, resulting in disrupted ATP production, cellular death, and ultimately, death of the organism [8].

Chlorfenapyr, which is a member of the pyrrole class of chemicals, is a proinsecticide compound; that is, its biological activity depends on its activation by another chemical. Oxidative removal of the N-ethoxymethyl group of Chlorfenapyr by mixed function oxidases forms uncouples oxidative phosphorylation at the mitochondria, resulting in disruption of production of ATP, cellular death, and ultimately organism mortality [3].

Monitoring of aquatic ecosystem pollution represents one of the major activities involved in measures aimed at environmental protection. Usage of non-targeted organisms in environmental toxicology is needed to understand the wide range of toxic effects caused by the pesticides on different organisms [23]. Fish and other aquatic biota that were commonly used as bioindicators of persistent organic pollutants [22] have been replaced in recent years successfully by ciliates [12]. Protozoan cells are often used as bioindicators of chemical pollution, especially in aquatic environment. The application of unicellular organisms to study the toxic effects of pesticides from contaminated wastewater is relatively new throughout the world. Hence, in the present paper, we have studied the toxic impacts of Chlorfenapyr a novel broad-spectrum insecticide-miticide, stable and persistent in the environment on oxidative stress in a pure culture of *Paramecium sp.*

**MATERIALS AND METHODS**

The biological material used is the typical representative of Protozoa: *Paramecium sp.* We kept a continuous pure colony in the laboratory at the University of Annaba from infusions of lettuce [1].
The pyrrole 4-Bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrol-3-carbonitrile is the common name of Chlorfenapyr that is used as an acaricide/insecticide and developed by Dow Agro-Sciences. This synthetic molecule was tested at the following concentrations: 250, 300 and 350 µM. These concentrations were selected after a series of preliminary finding range toxicity tests.

In accordance with Lavergne [9] and Sauvant, Pepin and Piccini [13], the growth kinetics of Paramecium sp. were studied by measuring the optical density OD at λ = 600 nm in function of time.

The response in percentage was calculated to evaluate the toxicity of xenobiotics via the inhibition of cell growth of protists. The percentage of positive values indicates an inhibition of growth, while the negative values indicate a stimulation of growth [26].

The polarographic method enables measurement of production or consumption of oxygen with an oxygen electrode, type HANSATECH [11].

The determination of glutathione (GSH) was performed as described by Weeckbeker and Cory [24] and glutathione S-transferase (GST) by Habig, Pabst and Jakoby [7]. These biomarkers were chosen for their role in cell protection and in cellular metabolism of xenobiotics. Measurements were made during the exponential phase of growth (between 48 and 96 h).

All the experiments were repeated three times, and the results were expressed as mean and standard error (SE) values. Statistical analysis was performed using a two-way ANOVA and the test of Dunnett for comparison between the control and treated cells. The α-level for significant differences was set at p<0.05 [5].

**Results:**

**Effect of Chlorfenapyr on the growth of Paramecium sp:**

The impact of Chlorfenapyr concentrations on the population growth of Paramecium sp. is shown in (Figure 1). The Chlorfenapyr has an inhibitory effect on cell growth of Paramecium (p<0.001), and this effect was already visible at 2nd day of the treatment.

**Fig. 1:** Effect of Chlorfenapyr at 250, 300 and 350 µM on the growth of Paramecium sp. Each data point represents the mean of three independent assays ± standard error. Values are significant from control values at p < 0.001.

**Percentage of response in Chlorfenapyr treated Paramecium sp:**

The response percentage measurement results are presented in (Figure 2). It can be said that the positive evolution of the response percentages confirm the growth inhibition of the treated paramecia and this regardless of the cell concentration.

**Fig. 2:** Percentage of response in Chlorfenapyr-treated Paramecium sp. Each value is mean ± standard error of three replicates.
Effect of Chlorfenapyr on GSH level in Paramecium sp:

As shown in figure 3, the GSH level is lower at the concentrations of 250 and 300 µM than in the non-treated Paramecium. In contrast, with the highest concentration (350 µM), the GSH level was increased significantly (p <0.001). We hypothesize that this increase results from the started detoxification/metabolism process.

![GSH level graph]

**Fig. 3:** Effect of Chlorfenapyr on the rate of GSH in *Paramecium* sp. Each value is mean ± standard error of three replicates.

Effect of Chlorfenapyr on GST activity in Paramecium sp:

As demonstrated in figure 4, that GST activity increased significantly in cells treated with different concentrations of Chlorfenapyr compared to controls (p <0.001).

![GST activity graph]

**Fig. 4:** Impact of Chlorfenapyr (250, 300 and 350 µM) on the GST activity in *Paramecium* sp. Each value is average±standard error of three independent observations.

Effect of Chlorfenapyr on the respiratory metabolism of Paramecium sp:

The O₂ consumption of paramecia was significantly affect (p<0.001) by the action of Chlorfenapyr concentrations (Figure 5). It should be noted that after 5 min of exposure, cell cultures treated with strongest concentration (350 µM) present a significantly deceleration of their respiratory activity.
Discussion and conclusions:

Paramecium that has long been a model organism for cellular aging and clonal lifespan [18,17,16,15,20,21]. In the present study, we showed that Chlorfenapyr concentrations molecule caused a dose-dependent growth inhibition of Paramecium sp. population. In addition, for the three concentrations tested, it can be said that the evolution of the response percentages confirm the growth inhibition of the treated Paramecium and this regardless of the cell concentration.

Low level of GSH and high GST activities recorded in the presence of xenobiotics may be indicative of activation of the detoxification system. On the other hand, as reported before [14,27,10,6] the direct capture of oxygen free radicals caused by xenobiotics is carried out by radical compounds as traps or enzyme systems located nearby the initial glutathione production system.

Chlorfenapyr is a member of a new class of chemicals -- the pyrroles. The compound is a pro-insecticide, i.e. the biological activity depends on its activation to another chemical. Oxidative removal of the N-ethoxymethyl group of Chlorfenapyr by mixed function oxidases forms uncouples oxidative phosphorylation at the mitochondria, resulting in disruption of production of ATP, cellular death, and ultimately organism mortality [3]. This inhibitory effect is reflected in our work by a metabolic disorder in the cellular respiration generated by an oxidative stress [4,25].

The results suggest that the antiproliferative effect of Chlorfenapyr may be mediated by reducing the chitin and cuticle of cells, also by the effect on mitochondria and lashes, our results allow us to conclude that the tested Chlorfenapyr inhibits the growth of Paramecium, and that this effect is mediated by a disruption of its cellular metabolism.

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

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