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Research Article
Effects of Cycloxydim on Population Growth, Phagocytosis, Contractile Vacuole Activity and Antioxidant Responses in the Freshwater Ciliate (*Paramecium tetraurelia*)

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Abstract
The continuous contamination of the aquatic environments by numerous agricultural and industrial pollutants has triggered the need to identifying new models and bioassays for assessing water pollution and understanding toxic effects of these xenobiotics at different levels. In the present study, investigation was done on physiological changes and antioxidant responses of the freshwater ciliate *Paramecium tetraurelia* exposed to a selective herbicide; Focus Ultra (a.i., cycloxydim: 100 g L^{-1}). Our results revealed an inhibitory effect on the cell proliferation rate. The growth inhibitory concentration (IC_{50}) was found to be 7.53 mg L^{-1}. Thus, the number and the velocity of generation were decreased when the generation time was extended significantly at the higher concentration of Focus Ultra (12 mg L^{-1}). The normal functioning of the contractile vacuole was also significantly affected at this concentration. Focus Ultra caused evenly a dose dependent stimulation of the phagocytic activity. Furthermore, monitoring biomarkers of oxidative stress showed a significant depletion in glutathione level (GSH) accompanied with a strong increase of glutathione S-transferase (GST) and catalase (CAT) activities reflecting the toxic effects of this herbicide on *Paramecium tetraurelia* cells.

Key words: Cycloxydim, *Paramecium tetraurelia*, phagocytic activity, oxidative stress, contractile vacuole

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The environmental pollution by dispersed chemical herbicides has become a serious problem especially in aquatic ecosystems due to their extensive use in agriculture and industry (Uri, 1999; Relyea, 2005). Although most herbicides are mainly applied to terrestrial environments, they can be found in diverse aquatic ecosystems albeit at highly variable concentrations (Carter, 2000; Relyea, 2005).

Graminicides belonging to the cyclohexanedione class, are post emergence herbicides widely used, to control annual and perennial grasses in a large variety of broad-leaved crop plants. They inhibit the enzyme, acetyl coenzyme A carboxylase (ACCase) and disrupt fatty acid biosynthesis in susceptible grasses (Burton *et al*., 1991; Devine and Shimabukuro, 1994). Cyclohexanedione herbicides are known to be biologically active at very low concentrations. Their polar character makes them easily leach to groundwater and potentially contaminant at levels above 0.1 μg L^{-1} (Sandin-Espana *et al*., 2003).

Cycloxydim is one of most popular cyclohexanediones. This active substance and its metabolites exhibits a high to very high mobility in soil and can potentially contaminate groundwater at levels reaching the 10 ng L^{-1} (Silva *et al*., 2006; EFSA., 2010). There are a number of studies carried out by EFSA (2010) on the toxicity of cycloxydim on non-target aquatic organisms. The acute toxicity data indicated that technical cycloxydim is harmful to aquatic organisms (EFSA., 2010).

The environmental disturbance of aquatic ecosystems has generated the need to develop methods for assessing the potential ecological effects of toxic chemicals on individual organisms, populations and communities. Recently, the use of biochemical processes as sensitive indicators of toxic stress had been paid much attention. Indeed, the continuous exposure of non-target organisms to pesticides may cause a metabolic imbalance, thus disrupting biochemical processes and leading to oxidative stress (Gowland *et al*., 2002; Jin *et al*., 2010). To minimize oxidative damage and harmful effects of Reactive Oxygen Species (ROS) to cellular components organisms are equipped with numerous defense mechanisms, (Di Giulio *et al*., 1995; Halliwell and Gutteridge, 2007), which can adapt to increasing ROS production by up-regulating antioxidant defenses, including the activities of enzymatic and non-enzymatic antioxidants (Livingstone, 2003).

The application of protists in laboratory researches as valuable bioassays offer the possibility for predicting toxic effects of chemical compounds (Garad *et al*., 2007; Fawole *et al*., 2008). Among protists, ciliated protozoa represent a basic component of aquatic environments (Jiang *et al*., 2013). Their microscopic size, ubiquitous distribution, high reproductive rate, relative ease of culturing and accessibility of experimental manipulation make of them excellent unicellular models for toxicological investigations (Morange, 2006; Weisse, 2006).

Paramecium is a ubiquitous freshwater ciliated protozoan that has been extensively investigated and increasingly being used as bioindicators in laboratory researchers to evaluating the environmental pollution and toxic effects of industrial, agricultural and domestic chemicals (Madoni, 2000; Miyoshi *et al*., 2003; Takahashi *et al*., 2005; Sbartai *et al*., 2009; Amanchi, 2010; Benbouzid *et al*., 2012). In this context, the main purpose of the present study is to evaluate physiological responses of the ciliated protozoan *Paramecium tetraurelia* exposed to a selective herbicide (cycloxydim). Inhibitory effects on growth and cell division were investigated by determining the IC_{50}. In addition, alterations caused by cycloxydim on phagocytic activity and contractile vacuole functioning of Paramecium cells were assessed. On the other hand, oxidative stress was monitored by measuring the antioxidant response using enzymatic (GST and CAT) and nonenzymatic (GSH) antioxidants.

MATERIALS AND METHODS

Chemicals: An emulsifiable concentrate of 100 g L^{-1} of cycloxydim (10.9%) (Fig. 1), was used in the present experiments as a test chemical, which is readily soluble in water. It is a selective herbicide used for outdoor foliar spraying against perennial grasses and commercialized under the name of Focus Ultra.

Paramecium culture and maintenance: *Paramecium tetraurelia* cells were cultured in a synthetic culture medium (pH 6.5) as described previously by Azzouz *et al*. (2011). The culture was maintained in a constant temperature of 30°C into the oven (memmert 400) and the cells were transplanted each three days in a new medium for keeping the youthful state of the culture.

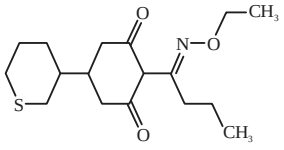

Fig. 1: Structural formula of the active ingredient (cycloxydim: 2-[1-(ethoxyimino)butyl]-3-hydroxy-5-(tetrahydro-2H-thiopyran-3-yl)-2-cyclohexen-1-one)

Treatment with the herbicide: Based on several preliminary assays carried out on different ranges of Focus Ultra, the appropriate stock solution and the test concentrations were selected. Indeed, four nominal test concentrations were chosen: 3, 6, 9 and 12 mg L^{-1}. The herbicide was tested in aliquots of 10 mL of culture medium and the treatment was done at the beginning (t = 0) before the transplantation of Paramecium cells. All experiments were performed with Paramecium cells in logarithmic growth phase. Thus, twenty active paramecia were exposed to selected concentrations with five replicates each.

Growth monitoring: The growth kinetics study was established by the daily cell counting during five days (120 h), after fixation with a Lugol solution at 1%, under optic microscope (LEICA DM 1000) using a striated slide. The count was repeated at least five times for each repetition (Azzouz *et al*., 2011).

Calculating the median inhibitory concentration, IC_{50}: The potential toxicity of Focus Ultra (cycloxydim) was quantified by determining the median inhibitory concentration (IC_{50}), which is the concentration required to induce 50% decrease in cell growth compared with the control cells for 96 h of treatment. Determination of the IC_{50} values was carried out by the kinetic growth method using the linear regression analysis.

Determination of the number, the time and the velocity of generation: The number of generations, the time required for each generation and the velocity of the generation were calculated by the following formulae:

$$\text{Number of generations} = \frac{\log N_t - \log N_0}{\log 2}$$

$$\text{Generation velocity} = \frac{\text{Number of generations}}{\text{Time of growth}}$$

$$\text{Generation time} = \frac{1}{\text{Generation velocity}}$$

where, N_t is the population in time t, N_0 is the initial number of cells.

Determination of the phagocytic activity: Phagocytic activity was estimated by a staining technique suggested by Rouabhi *et al*. (2006). It consists to mix a drop of 1% of neutral red with a drop of culture sample (control or treated cells). After 3 min, cells were placed between a glass slide and a coverslip and the total number of labelled food vacuoles per cell was counted under optic microscope type LEICA DM 1000 (×20 objective).

Measurement of expulsion frequency of the contractile vacuole: To restrict the movement of Paramecium, cells were slightly compressed between a glass slide and a coverslip as proposed by Naitoh *et al*. (1997). Expulsion frequencies were video-recorded (Leica EC 3) under optic microscope (×40 objective). The rate of pulsation per minute of the anterior contractile vacuole (from the beginning of one contraction to the beginning of the next one) was determined for the control and treated groups. Five readings were taken for each cell.

Biochemical assays: For biomarkers analysis, treatment was done in the fourth day of culture (the end of exponential phase) to avoid any influence on growth. The beginning of the assay for each biomarker takes place three hours after treatment with the herbicide. A preconcentration of Paramecium cells at 3000 rpm min^{-1} is necessary. Then, the pellet is recovered for use in the assay of glutathione (GSH), glutahtion-S-transferase (GST) and catalase (CAT). All assays were performed in triplicate (Azzouz, 2012).

Determination of glutathione rate (GSH): The rate of GSH was quantified according to the method of Weckberker and Cory (1988). Cells were homogenized in 1 mL of EDTA (0.02 M) and 0.2 mL of sulfosalicylic acid (ASS) was added to 0.8 mL of homogenate. After agitation, the homogenate was centrifuged at 1000 rpm for 5 min. The reaction was initiated by adding to 0.5 mL of supernatant, 1 mL of tris/EDTA buffer (0.02 M, pH 9.6) and 0.025 mL of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). The absorbance was measured at 412 nm and the amount of GSH was expressed as µmol mg^{-1} of proteins.

Measurement of glutahtion-S-transferase activity (GST): Determination of GST activity was performed by the method of Habig *et al*. (1974) by following the conjugation of GSH with CDNB. After homogenization of the sample in 1 mL of phosphate buffer (0.1M, pH 6), homogenate was centrifuged at 14000 rpm for 30 min. 1.2 mL of the mixture CDNB (1 mM)/GSH (1mM) was added to 0.2 mL of supernatant to start the reaction. The formation of S-2,4-dinitrophenyl glutathione conjugate was evaluated by recording the increase in absorbance at 340 nm for 5 min after every 1 min interval. It was expressed as µmol/min/mg protein.

Measurement of catalase activity (CAT): The CAT activity was measured following the method of Claiborne (1985) whose

the principle is based on the change in optical density due to the dismutation of hydrogen peroxide (H_2O_2). Samples were homogenized in 1mL of phosphate buffer (100 mM, pH 7.5) then centrifuged at 15000 g for 10 min. The 0.75 mL of phosphate buffer and 0.2 mL of H_2O_2 were added to 0.050 mL of the supernatant. Decrease in absorbance caused by H_2O_2 consumption was monitored at 240 nm for 30 sec with an interval of 15 sec. The results of this enzymatic assay were expressed as µmol/min/mg protein.

Statistical analysis: Experiments were carried out at least in triplicate and all values were expressed as the Mean±Standard Deviation (SD). To evaluate the relationship between the concentrations of Focus Ultra and the effects on the growth we used the regression analysis. Data were analyzed by one-way analysis of variance (ANOVA). To look for differences by pairs between treated samples and the control, we applied the Student's t-test and a value of $p \leq 0.05$ was considered statically significant. Statistical analyses were performed using the Minitab software (14.0) (Dagnelie, 1999).

RESULTS

Effects of Focus Ultra on the growth rate of *Paramecium tetraurelia*: Focus Ultra showed a significant inhibitory effect on the population density of *P. tetraurelia* in a concentration dependent manner and the growth rate was reduced significantly with the increasing of Focus Ultra concentrations (Fig. 2).

In the first 24 h of exposure, no significant changes were observed in the growth rate of paramecia treated by the lowest concentrations (3 and 6 mg L^{-1}) of the herbicide when compared to the control ($p>0.05$). However, a highly significant depletion in cell number was noticed, since the third day of exposure (72 h), with the highest concentrations (9 and 12 mg L^{-1}) of Focus Ultra ($p \leq 0.001$). Thus, it caused about 50 and 70% of the growth decrease, respectively.

Calculating the IC_{50}: According to the growth curve, the IC_{50}, causing a 50% decrease in growth rate, was calculated from the linear equation "Y = 7.963x – 10" to be 7.53 mg L^{-1} (Fig. 3).

Effects on the number, the time and the velocity of generation: Figure 4 illustrates the effect of Focus Ultra on the number, the time and the velocity of generation. The results showed that Focus Ultra has gradually affected the multiplication of *P. tetraurelia* and led to a slower growth and a delayed cell division.

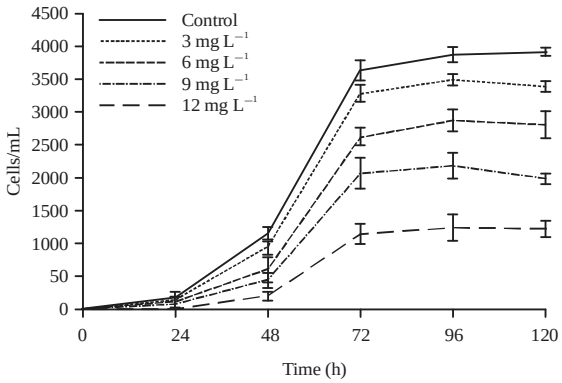

Fig. 2: Growth kinetics of *P. tetraurelia* exposed to different concentrations of Focus Ultra

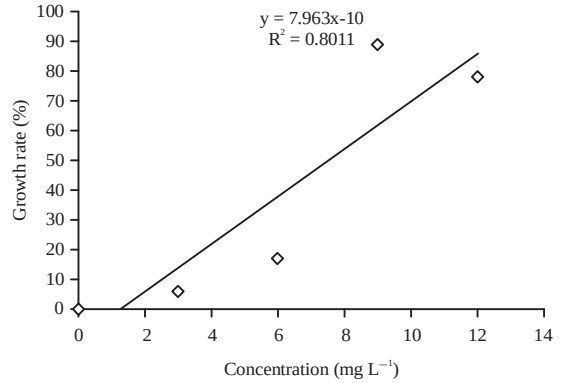

Fig. 3: Determination of the IC_{50} by the kinetic growth method

Table 1: Phagocytic activity of *P. tetraurelia* after treatment with Focus Ultra

Concentrations of Focus Ultra (mg L^{-1})	Number of food vacuoles per cell
Control	9.80±1.92*
3	15.0±1.41*
6	16.4±2.07*
9	20.4±2.07*
12	28.2±2.86*

Values are represented as Mean±SD (n = 5). Significant differences from the control are indicated as: *$p \leq 0.05$

Exposure to increasing concentrations of Focus Ultra caused an increase of the generation time in a dose dependent manner (Fig. 4b). The generation time of the control *P. tetraurelia* cells was calculated to be 12.6 h; however, it increased at the higher concentration (12 mg L^{-1}) to reach approximately 16 h. Consequently, the number and the velocity of generation were inversely proportional to the concentrations of Focus Ultra and they knew a progressive decrease along with increasing concentrations (Fig. 4a and c).

Phagocytic activity: Results concerning the effect of Focus Ultra on phagocytic activity in *P. tetraurelia* are illustrated in Table 1. A dose-dependent stimulatory effect of the number of food vacuoles in cells treated with increased concentrations

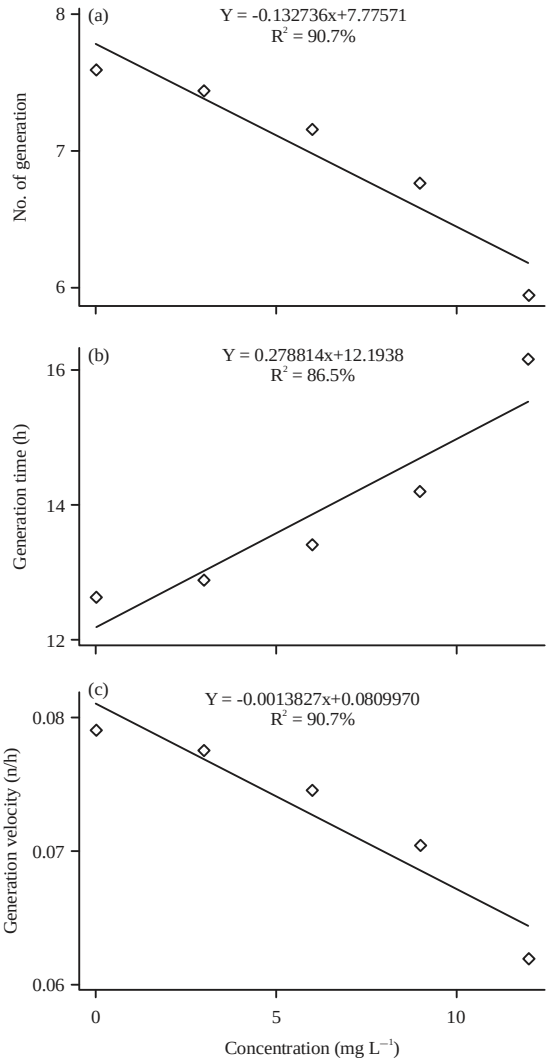

Fig. 4(a-c): Effects of Focus Ultra on (a) The number of generation (b) Generation time and (c) Velocity of generation of *P. tetraurelia*

Table 2: Contractile vacuole activity in *P. tetraurelia* exposed to increased concentrations of Focus Ultra

Concentrations of Focus Ultra (mg L^{-1})	No. of pulses min^{-1}	Average time of one pulsation (sec)
Control	12.3±1.56	4.88±0.70
3	11.8±1.64	5.08±0.85
6	10.2±2.77	5.88±1.20
9	9.4±2.88	6.38±1.22
12	7.6±1.93*	7.90±0.86

Values are represented as Mean±SD (n = 5). Significant differences from the control are indicated as: *p≤0.05

of Focus Ultra compared to the control was observed (Fig. 5). Indeed, the average number of food vacuoles was significantly elevated from 9.8 in control cells to 15 and 16.4 in paramecia exposed to 3 and 6 mg L^{-1} (p = 0.002 and p = 0.001), respectively. The maximal stimulation was occurred in cells treated with the highest concentrations (9 and 12 mg L^{-1}) of Focus Ultra, noted values were equal to 20.4 and 28.2 food vacuoles/cells (p = 0.000).

Contractile vacuole activity: As shown in Table 2, a dose dependent decrease in expulsion frequency of the contractile vacuole was found in Paramecium cells exposed to Focus Ultra. In concentration 3, 6 and 9 mg L^{-1}, the vacuolar activity was insignificantly diminished when compared to the control. The maximum inhibitory effect on contractile vacuole activity was recorded at the highest concentration of 12 mg L^{-1}. In fact, a significant vacuolar retardation estimated by 7.6 pulses min^{-1} (p = 0.006) and an average time of 7.9 sec for one pulsation, as compared with control values of 12.3 pulses min^{-1} and 4.88 sec for one pulsation was noted.

Antioxidant responses: The results of antioxidant enzyme activities (CAT and GST) and the GSH level are presented in Fig. 6.

A significant increase in CAT activity compared with the control was observed following exposure to all concentrations of Focus Ultra (p = 0.000), except for the lowest concentration of 3 mg L^{-1} (p = 0.775) (Fig. 6a). Thus, it was from 11.93±1.63 μM/min/mg protein and 17.22±3 μmol/min/mg protein for the concentrations (6 and 9 mg L^{-1}), respectively and reached a rate of 24.23±1.17 μmol/min/mg protein for the highest concentration of 12 mg L^{-1}. While, in control cells catalase activity was only from 4.75±0.41 μmol/min/mg protein.

On the other hand, exposure of *P. tetraurelia* to this herbicide resulted in a strong induction of the GST activity (Fig. 6b). The greatest enzymatic activity appeared with concentrations (9 and 12 mg L^{-1}), which had highly significant increase when compared to the control (p = 0.004 and p = 0.008). However, the effects of Focus Ultra were less intense in concentrations 3 and 6 mg L^{-1}, where there were no significant differences in GST activity (p = 0.434 and p = 0.129).

The results regarding the variation of the GSH level are shown in Fig. 6c. It was found that Focus Ultra causes a dose dependent decrease in the GSH rate. Compared to the control (18.05±2.52), GSH level was significantly reduced to be 10.47±0.85 μmol mg^{-1} protein (p = 0.039) in paramecia treated with the concentration of 6 mg L^{-1}. For cells treated with 9 and 12 mg L^{-1} this rate was determinate to be 7.99±1.54 and 4.47±1.18 μmol mg^{-1} protein (p = 0.010 and p = 0.014), respectively.

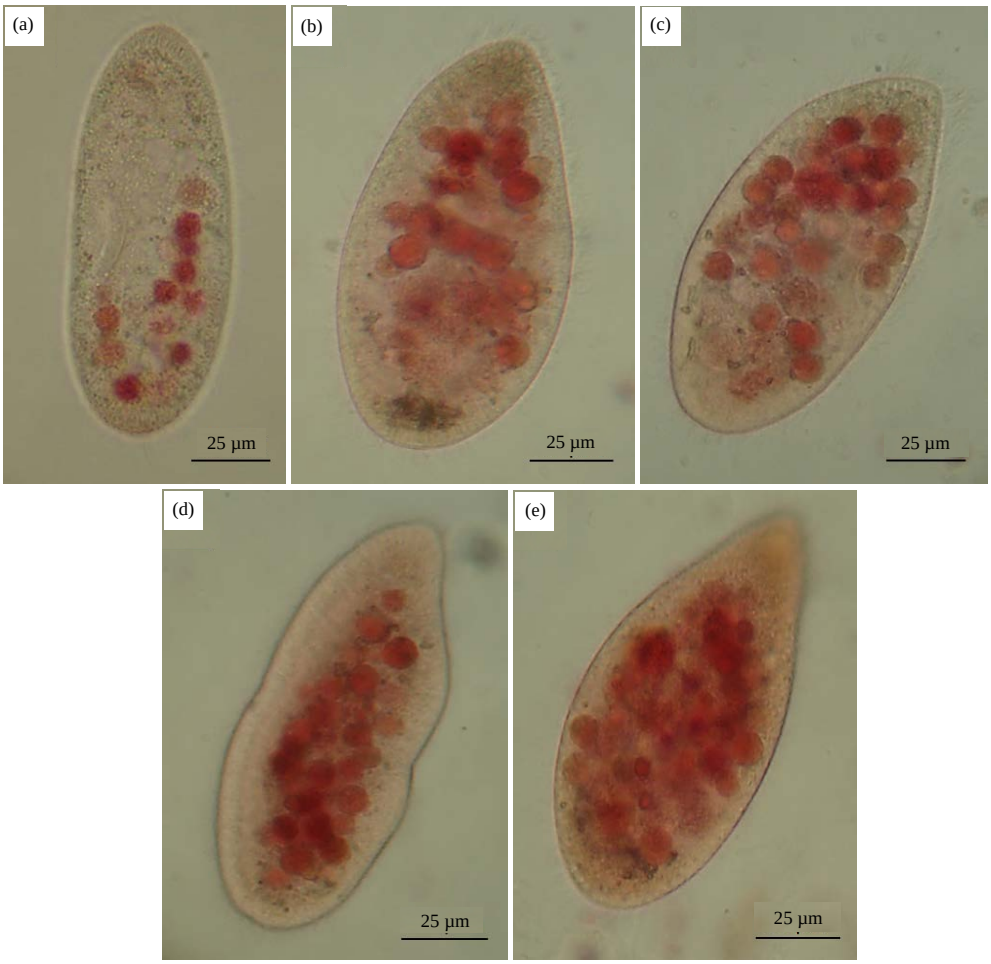

Fig. 5(a-e): Microscopic images of (a) *P. tetraurelia* control, (b) Exposed cells to 3 mg L^{-1}, (c) 6 mg L^{-1}, (d) 9 mg L^{-1} and (e) 12 mg L^{-1} of Focus Ultra, strained with neutral red at 1%

DISCUSSION

Due to their high degree of selectivity and efficiency against both annual and perennial grass weeds, cyclohexanediones are important herbicides in crop production (Kukorelli *et al*., 2013). However, their intensive use may cause many toxicological and environmental problems and has led to the emergence of herbicide resistant weeds (Toyama *et al*., 2003; Hochberg *et al*., 2009; Collavo *et al*., 2011). This could result in significant economic losses and the necessity of using higher herbicide rates with deleterious consequences to both the environment and human health (Park *et al*., 2010).

Cycloxydim is a frequently used active ingredient from the chemical group of cyclohexanediones, which have been qualified as high-risk herbicide (Mohassel *et al*., 2010). Several studies have been carried out on the efficiency of cycloxydim on a wide variety of troublesome weeds and their resistance to this herbicide (Delye *et al*., 2002; Mohassel *et al*., 2010; Cruz-Hipolito *et al*., 2012). Although, microorganisms are convenient for culture and highly sensitive to many chemical substances (Weisse, 2006), the toxicological effects of cycloxydim on microorganisms, especially ciliates have not been well documented. Majority of studies have been conducted on plants. From this vision, the aim of the present study is to evaluate the toxicity of cylcoxydim on a ciliate protozoan: *Paramecium tetraurelia*, by monitoring some physiological and antioxidant responses of cells exposed to the commercial formulation of Focus Ultra.

Concerning physiological responses, our results demonstrated that the toxicity of Focus Ultra was expressed by an inhibition of the cell growth with an IC_{50} value of about 7.53 mg L^{-1}. Further, it induced an inhibition effect on cellular division. This inhibition provokes an important increase in

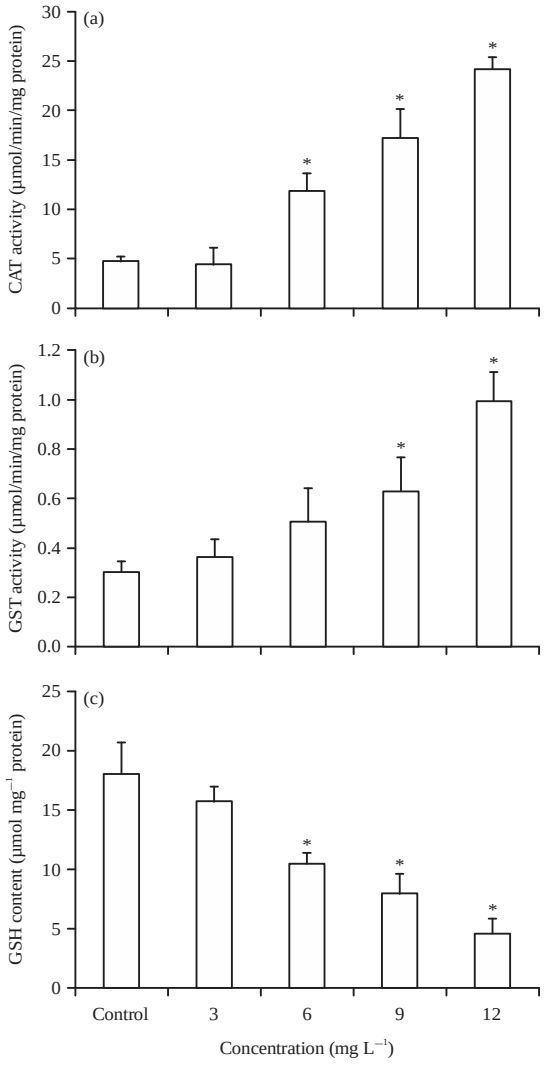

Fig. 6(a-c): Variation of oxidative stress biomarkers depending on different concentrations of Focus Ultra (a) CAT activity, (b) GST activity and (c) GSH rate. Significant differences from the control are indicated as: *p≤0.05

generation time with a decrease of the number and the velocity of generation. This can be explained by the fact that increasing concentrations of toxicants in the cell may affect survival and cellular division of protists thereby causing cell death (Madoni, 2000; Mountassif *et al*., 2007). Ma et *al*. (2006) also studied the acute toxicity of 40 herbicides with nine modes of action on the freshwater unicellular green algae *Raphidocelis subcapitata* and pointed out that acetyl CoA carboxylase inhibitors (clethodim, diclofop-P, fenoxaprop, fluazifop-P, haloxyfop-R and quizalofop-P), which have the same mode of action of our experimental herbicide (cycloxydim) occupied the third place in the descending order of the average acute toxicity, with a 96 h EC_{50} range of 0.2-5.3 mg L^{-1}. Similarly, the results are in accordance with those of Azzouz *et al*. (2011) and Rao *et al*. (2006) that demonstrated an inhibition in cell growth and proliferation of paramecia exposed to increased concentrations of amistar xtra and acephate, respectively. Recently, the same results were also observed by Shubhamsingh and Tejashree (2014).

A dose dependent stimulatory effect of the rate of phagocytosis was also ascertained during treatment with Focus Ultra. Thus, our results showed a significant increase in the number of food vacuoles along with increasing concentrations of the herbicide. It have been demonstrated previously that formation and movement of food vacuoles in the cell is assured by ciliary motility. In fact, factors affecting the ciliary action should necessarily influence the rate of food vacuole formation (Nilsson, 2003). The results are consistent with those of Nilsson (1981) that noted a stimulation of phagocytosis in Tetrahymena cells treated with increased concentrations of copper and attributed this increase to a possible correlation with increasing cell motility. This is the case of the present study where an increase in swimming velocity of Paramecium cells exposed to Focus Ultra was observed. These finding can be also explained by the fact that the presence of a high content of organic matter in the culture medium can increase the tolerance of ciliates to higher concentrations of Focus Ultra, as it was shown earlier by Nilsson (1981). Stefanidou *et al*. (1990) reported evenly a stimulation in the phagocytic ability of protozoans exposed to amphetamine and interpreted this result by a probable increase in the energy level caused by the drug. In addition, Amanchi and Hussain (2008) suggested that the presence of pesticides in the external environment of the cell can modify the pH of the medium and consequently change the food vacuole formation. A disturbance of the phagocytosis was similarly discerned by Nilsson (2005) in *Tetrahymena pyriformis* treated with cigarette extracts and ethanol, respectively. On the other hand, the results disagreed with those of Jaleel (2002), Hussain *et al*. (2008) and Amanchi and Hussain (2012). These authors pointed out that the inhibition of phagocytosis could be due to a damage in cell membrane or cilia structure.

Osmoregulation in Paramecium is provided by the activity of the Contractile Vacuole Complex (CVC) and depended on both regulatory mechanisms controlling the cytosolic osmolarity and water permeability of the plasma membrane (Stock *et al*., 2001). The contractile vacuole was one of the first organelles to be visualized within Paramecium and has stimulated more research than any other protozoan organelle, beginning with the earliest microscopists, in order

to understand the structure and functioning mechanisms of this complex (Patterson, 1980). It have been previously agreed that contractile vacuole complex of ciliates is very highly sensitive to changes in the cell's environment and the frequency of expulsion can be modified, particularly, by the osmotic pressure of the external medium (Patterson, 1980). Effectively, the addition of Focus Ultra to the culture medium has caused a dose dependent decrease in vacuolar activity of *P. tetraurelia*. This can be a response to the sudden change in the external osmolality as it was described already by Amanchi and Hussain (2008). They suggested evenly that disruption of the vacuolar apparatus can be a probable raison to the decrease of contractile vacuole activity. Recently, Shubhamsingh and Tejashree (2014) reported also that the insecticide dimethoate has an inhibitory effect on the contractile vacuole complex of *Paramecium* sp.

In environmental studies, biomarkers were considered as early warning signals usually employed in the field of risk assessment and biomonitoring to identify and predict effects of toxicants on biological organisms (Van der Oost *et al*., 2003; Jemec *et al*., 2012). Thus, the use of biomarkers of oxidative stress provides detailed information on the potential toxicity of pollutants (Barata *et al*., 2005).

In this study, interest was focused evenly on antioxidant responses of *P. tetraurelia* in the presence of Focus Ultra. Results revealed a strong antioxidant enzyme activity resulting in an increase of GST and CAT activities, known for their role in the detoxification of free radicals, parallel to the decrease of GSH level which is considered as an excellent biomarker of toxicity. These enzymatic and non-enzymatic antioxidant reactions provide to the cell a state of equilibrium and protection against oxidized reactive species (Mofredj *et al*., 1999).

As the vital first-line defenses against oxidative stress, CAT is a key antioxidant enzyme mainly located in peroxisomes (Yu, 1994) and designed to reducing hydrogen peroxide resulting from the metabolism of long chain fatty acids (Yi *et al*., 2007). In the present study, CAT activity was significantly increased at higher concentrations of Focus Ultra. This increase might be contributed to the elimination from the cell of Reactive Oxygen Species (ROS) induced by pesticide exposure, which converts oxygen free radical (O_2^-) into H_2O_2 and then into H_2O and O_2 (Barata *et al*., 2005; Jin *et al*., 2010). These results are supported by the findings of Bouaricha (2013) and Sbartai (2013) who recorded an intensification of CAT activity in Paramecium cells treated with bifenazate and proclaim. According to Yu *et al*. (2009), the higher enzyme activity could efficiently protect against potential increased production of ROS and may reflect an adaptation in response to the oxidative conditions to which freshwater organisms are exposed.

At the cellular level, the antioxidant defense system consisting of both small molecule free radical scavengers and antioxidant enzymes has as function the detoxification and elimination of ROS. Glutathione S-transferase is just one of the multicomponent enzymes interfered in the detoxification of different toxicants and protecting tissues from oxidative damage (Khurana *et al*., 2002). His activity was notified to be upregulated by induction of the antioxidant/electrophile responsive element during oxidative stress (Ahlgren-Beckendorf *et al*., 1999). In our investigation, a higher GST activity was observed following exposure to most of the concentrations of Focus Ultra. This can be understood in view of the fact that pesticides consume GSH through a GST-catalyzed reaction as a major way of detoxification and these chemicals are expected to induce the activity of GST as a potent protection mechanism of the organism (Timur *et al*., 2002). Similarly, GST was significantly induced in *P. tetraurelia* cells exposed to the highest concentration of cypermethrin insecticide (Amamra *et al*., 2015).

The GSH and GSH-related enzymes are considered as the vital second-line defenses against oxidative damage and play a major role in cellular metabolism and free radical scavenging (Pena-Llopis *et al*., 2003; Liu *et al*., 2008). The marked decline in GSH level in the present work might be due to its massive utilization to challenge the prevailing oxidative stress resulting from excess generation of ROS (Ranjbar *et al*., 2005; Ansari and Ansari, 2014). Likewise, the depletion of GSH level may be attributed to detoxification process depending on glutathione-S-transferase (GST) enzyme which is one of the enzyme systems that consuming GSH molecules as a substrate (Farag *et al*., 2010). The experimental results are in agreement with earlier studies (Grara *et al*., 2012; Saib *et al*., 2014) that elucidated a significant depletion of the GSH content in paramecia exposed to polymethyl methacrylate (dental resin) and amidophosphonate (organophosphorus insecticide), respectively.

CONCLUSION

Based on experimental data obtained throughout this study, it appears that *Paramecium tetraurelia* cells are sensitive to cycloxydim this sensitivity is manifested by a disturbance in the physiological state of Paramecium cells, accompanied with a global change in responses of antioxidant biomarkers. Given their sensitivity and particular cellular organization, it was confirmed that these ciliates are ideal alternative models for biomonitoring programs and assessing chemical toxicity. However, further detailed studies should be focused on better understanding the cycloxydim mechanism of action, as well as

evaluating his genotoxic effects in Paramecium cells. It would also be interesting to explore the long-term effects of this herbicide or else test it on other aquatic organisms.

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REFERENCES

Ahlgren-Beckendorf, J.A., A.M. Reising, M.A. Schander, J.W. Herdler and J.A. Johnson, 1999. Coordinate regulation of NAD(P)H:Quinone oxidoreductase and glutathione-S-transferases in primary cultures of rat neurons and glia: Role of the antioxidant/electrophile responsive element. Glia, 25: 131-142.

Amamra, R., M.R. Djebar, N. Grara, O. Moumeni, H. Otmani, A. Alayat and H. Berrebbah, 2015. Cypermethrin-induces oxidative stress to the freshwater ciliate model: *Paramecium tetraurelia*. Annu. Res. Rev. Biol., 5: 385-399.

Amanchi, N.R. and M.M. Hussain, 2008. Cytotoxic effects of delfin insecticide (*Bacillus thuringiensis*) on cell behaviour, phagocytosis, contractile vacuole activity and macronucleus in a protozoan ciliate *Paramecium caudatum*. Afr. J. Biotechnol., 7: 2637-2643.

Amanchi, N.R., 2010. A low cost microbiotest for screening behavioural and ecotoxicological responses of *Paramecium caudatum* and *Oxytricha fallax* to azadirachtin. Adv. Appl. Sci. Res., 1: 124-131.

Amanchi, N.R. and M.M. Hussain, 2012. Ecophysiological and cytopathological impact of delfin insecticide (*Bacillus thuringiensis*) to an unicellular ciliate protozoan, *Euplotes patella*. Res. J. Recent Sci., 1: 64-67.

Ansari, S. and B.A. Ansari, 2014. Temporal variations of CAT, GSH and LPO in gills and livers of zebrafish, *Danio rerio*, exposed to dimethoate. Arch. Polish Fish., 22: 101-109.

Azzouz, Z., H. Berrebbah and M.R. Djebar, 2011. Optimization of *Paramecium tetraurelia* growth kinetics and its sensitivity to combined effects of azoxystrobin and cyproconazole. Afr. J. Microbiol. Res., 5: 3243-3250.

Azzouz, Z., 2012. Study of toxic effects of a fungicide (Amistar Xtra) and a herbicide (Glyphosate) on the biology and comportment of *Paramecium tetraurelia*. Ph.D. Dissertation Badji Mokhtar University, Annaba, Algeria.

Barata, C., I. Varo, J.C. Navarro, S. Arun and C. Porte, 2005. Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 140: 175-186.

Benbouzid, H., H. Berrebbah, M. Berredjem and M.R. Djebar, 2012. Toxic effects of phosphoramidate on *Paramecium* sp. with special emphasis on respiratory metabolism, growth and generation time. Toxicol. Environ. Chem., 94: 557-565.

Bouaricha, H., 2013. Evaluation of oxidative stress induced by Proclaim: Comparative test on two biological models (*Helix aspersa* and *Paramecium* sp.). Ph.D. Dissertation, Badji Mokhtar University, Annaba, Algeria.

Burton, J.D., J.W. Gronwald, R.A. Keith, D.A. Somers, B.G. Gengenbach and D.L. Wyse, 1991. Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Pestic. Biochem. Physiol., 39: 100-109.

Carter, A., 2000. How pesticides get into water-and proposed reduction measures. Pestic. Outlook., 11: 149-156.

Claiborne, A., 1985. Catalase Activity. In: CRC Handbook of Methods for Oxygen Radical Research, Greenwald, R.A. (Ed.). 3rd Edn., CRC Press, Boca Raton, FL., USA., ISBN-13: 9780849329364 pp: 283-284.

Collavo, A., S. Panozzo, G. Lucchesi, L. Scarabel and M. Sattin, 2011. Characterisation and management of *Phalaris paradoxa* resistant to ACCase-inhibitors. Crop Protect., 30: 293-299.

Cruz-Hipolito, H., J.A. Dominguez-Valenzuela, M.D. Osuna and R. De Prado, 2012. Resistance mechanism to acetyl coenzyme A carboxylase inhibiting herbicides in *Phalaris paradoxa* collected in Mexican wheat fields. Plant Soil, 355: 121-130.

Dagnelie, P., 1999. Theoretical and Applied Statistics: Statistical References to One and Two Dimensional. University of Minnesota Press, Minneapolis pages: 659.

Delye, C., E. Calmes and A. Matejicek, 2002. SNP markers for black-grass (*Alopecurus myosuroides* Huds.) genotypes resistant to acetyl CoA-carboxylase inhibiting herbicides. Theoret. Applied Genet., 104: 1114-1120.

Devine, M.D. and R.H. Shimabukuro, 1994. Resistance to Acetyl Coenzyme A Carboxylase Inhibiting Herbicides. In: Herbicide Resistance in Plants: Biology and Biochemistry, Powles, S.B. and J.A.M. Holtum (Eds.). Chapter 5, Lewis Publishers, Boca Raton, FL., USA. ISBN 13: 9780873717137 pp: 141-169.

Di Giulio, R.T., W.H. Benson, B.M. Sanders and P.A. Van Veld, 1995. Biochemical Mechanisms: Metabolism, Adaptation and Toxicity. In: Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment, Rand, G.M. (Ed.). 2nd Edn., Chapter 17, Taylor and Francis Inc., Boca Raton, FL., USA., ISBN-13: 978-1560320913, pp: 523-562.

EFSA., 2010. Conclusion on the peer review of the pesticide risk assessment of the active substance cycloxydim. EFSA J., Vol. 8, No. 7. 10.2903/j.efsa.2010.1669

Farag, A.G.A., M.E.A. Elhalwagy and H.E.A. Farid, 2010. Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats. Pestic. Biochem. Physiol., 97: 267-274.

Fawole, O.O., T.A. Yekeen, A.A. Ayandele, A. Akinboro, M.A. Azeez and S.O. Adewoye, 2008. Polluted Alamuyo River: Impacts on surrounding wells, microbial attributes and toxic effects on *Allium cepa* root cells. Asian J. Biotechnol., 7: 450-458.

Garad, U., S.N. Desai and P.V. Desai, 2007. Toxic effects of monocrotophos on *Paramecium caudatum*. Afri. J. Biotechnol., 6: 2245-2250.

Gowland, B.T.G., C.F. Moffat, R.M. Stagg, D.F. Houlihan and I.M. Davies, 2002. Cypermethrin induces glutathione S-transferase activity in the shore crab, *Carcinus maenas*. Mar. Environ. Res., 54: 169-177.

Grara, N., B. Houda, B. Mounir, A. Amira, B. Houria, D.M. Reda and Z. Chafica, 2012. Evaluation of the potential toxicity of the polymethyl methacrylate (dental resin) on a cellular model alternate Paramecium. Adv. Environ. Biol., 6: 1392-1399.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione *S*-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Halliwell, B. and J.M.C. Gutteridge, 2007. Free Radicals in Biology and Medicine. 4th Edn., Clarendon Press, Oxford, UK., ISBN-13: 9780198568698, Pages: 888.

Hochberg, O., M. Sibony and B. Rubin, 2009. The response of ACCase-resistant *Phalaris paradoxa* populations involves two different target site mutations. Weed Res., 49: 37-46.

Hussain, M.M., N.R. Amanchi, V.R. Solanki and M. Bhagavathi, 2008. Low cost microbioassay test for assessing cytopathological and physiological responses of ciliate model *Paramecium caudatum* to carbofuran pesticide. Pestic. Biochem. Physiol., 90: 66-70.

Jaleel, M.A., 2002. *In vitro* toxicity evaluation of some pesticides using certain protozoa. Ph.D. Dissertation, Osmania University, Hyderabad, India.

Jemec, A., T. Tisler, B. Erjavec and A. Pintar, 2012. Antioxidant responses and whole-organism changes in *Daphnia magna* acutely and chronically exposed to endocrine disruptor bisphenol A. Ecotoxicol. Environ. Saf., 86: 213-218.

Jiang, Y., H. Xu, M. Zhu and K.A.S. Al-Rasheid, 2013. Temporal distributions of microplankton populations and relationships to environmental conditions in Jiaozhou Bay, Northern China. J. Mar. Biol. Assoc. UK., 93: 13-26.

Jin, Y., X. Zhang, L. Shu, L. Chen and L. Sun *et al*., 2010. Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*). Chemosphere, 78: 846-852.

Khurana, S., M.T. Corbally, F. Manning, T. Armenise, B. Kierce and C. Kilty, 2002. Glutathione S-transferase: A potential new marker of intestinal ischemia. J. Pediatr. Surg., 37: 1543-1548.

Kukorelli, G., P. Reisinger and G. Pinke, 2013. ACCase inhibitor herbicides-selectivity, weed resistance and fitness cost: A review. Int. J. Pest Manage., 59: 165-173.

Liu, Y., J. Wang, Y. Wei, H. Zhang, M. Xu and J. Dai, 2008. Induction of time-dependent oxidative stress and related transcriptional effects of perfluorododecanoic acid in zebrafish liver. Aquatic Toxicol., 89: 242-250.

Livingstone, D.R., 2003. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Revue Medecine Veterinaire, 154: 427-430.

Ma, J., S. Wang, P. Wang, L. Ma, X. Chen and R. Xu, 2006. Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*. Ecotoxicol. Environ. Saf., 63: 456-462.

Madoni, P., 2000. The acute toxicity of nickel to freshwater ciliates. Environ. Pollut., 109: 53-59.

Miyoshi, N., T. Kawano, M. Tanaka, T. Kadono and T. Kosaka, 2003. Use of *Paramecium* species in bioassays for environmental risk management: Determination of IC_{50} values for water pollutants. J. Health Sci., 49: 429-435.

Mofredj, A., J.F. Cadranel, B. Darchy, J.C. Barbare, A. Cazier, V. Pras and M. Biour, 1999. [Hepatotoxicity caused by therapeutic doses of paracetamol in alcoholics. Report of 2 cases of fatal hepatitis in cirrhosis]. Annales Medecine Interne, 150: 507-511, (In French).

Mohassel, M.H.R., A. Aliverdi, H. Hamami and E. Zand, 2010. Optimizing the performance of diclofop-methyl, cycloxydim and clodinafop-propargyl on littleseed canarygrass (*Phalaris minor*) and wild oat (*Avena ludoviciana*) control with adjuvants. Weed Biol. Manage., 10: 57-63.

Morange, M., 2006. What history tells us IV. Ciliates as models of what? J. Biosci., 31: 27-30.

Mountassif, D., M. Kabine, R. Manar, N. Bourhim, Z. Zaroual, N. Latruffe and M.H.S. El Kebbaj, 2007. Physiological, morphological and metabolic changes in *Tetrahymena pyriformis* for the in vivo cytotoxicity assessment of metallic pollution: Impact on D-β-hydroxybutyrate dehydrogenase. Ecol. Indicators, 7: 882-894.

Naitoh, Y., T. Tominaga and R.D. Allen, 1997. The contractile vacuole fluid discharge rate is determined by the vacuole size immediately before the start of discharge in *Paramecium multimicronucleatum*. J. Exp. Biol., 200: 1737-1744.

Nilsson, J.R., 1981. Effects of copper on phagocytosis in Tetrahymena. Protoplasma, 109: 359-370.

Nilsson, J.R., 2003. Lanthanum affects proliferation and motility of Tetrahymena. Eur. J. Protistol., 39: 468-474.

Nilsson, J.R., 2005. Ethanol affects endocytosis and proliferation of *Tetrahymena pyriformis* GL and promotes encystment. Acta Protozoologica, 44: 293-299.

Park, K., J. Park, J. Kim and I.S. Kwak, 2010. Biological and molecular responses of *Chironomus riparius* (Diptera, Chironomidae) to herbicide 2,4-D (2,4-dichlorophenoxyacetic acid). Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 151: 439-446.

Patterson, D.J., 1980. Contractile vacuoles and associated structures: Their organization and function. Biol. Rev., 55: 1-46.

Pena-Llopis, S., M.D. Ferrando and J.B. Pena, 2003. Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by *N*-acetylcysteine. Aquat. Toxicol., 65: 337-360.

Ranjbar, A., H. Solhi, F.J. Mashayekhi, A. Susanabdi, A. Rezaie and M. Abdollahi, 2005. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. Environ. Toxicol. Pharmacol., 20: 88-91.

Rao, J.V., K. Srikanth, S.K. Arepalli and V.G. Gunda, 2006. Toxic effects of acephate on *Paramecium caudatum* with special emphasis on morphology, behaviour and generation time. Pest. Biochem. Physiol., 86: 131-137.

Relyea, R.A., 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Applic., 15: 618-627.

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: 45-48.

Saib, A., H. Berrebbah, M. Berredjem and M.R. Djebar, 2014. Cytotoxic study of three derivatives amidophosphonates on alternative cellular model: *Paramecium tetraurelia*. Toxicol. Res., 3: 395-399.

Sandin-Espana, P., S. Llanos, J.O. Magrans, J.L. Alonso-Prados and J.M. Garcia-Baudin, 2003. Optimization of hydroponic bioassay for herbicide tepraloxydim by using water free from chlorine. Weed Res., 43: 451-457.

Sbartai, I., H. Berrebbah, R. Rouabhi, H. Sbartai, S. Guy and M.R. Djebar, 2009. Behavior of *Paramecium* sp., treated with bifenazate with special emphasis on respiratory metabolism, protein and generation time. Am.-Eurasian J. Toxicol. Sci., 1: 13-18.

Sbartai, I., 2013. Toxicity of a hydrazine carboxylate (Bifenazte) and an oxadiazine (Indoxacarb) observed in a cellular freshwater model: *Paramecium* sp. Ph.D. Dissertation, Badji Mokhtar University, Annaba, Algeria.

Shubhamsingh, T. and S. Tejashree, 2014. Effect of neurotoxic insecticide dimethoate on unicellular freshwater protozoan ciliate *Paramecium* sp. Int. Res. J. Biol. Sci., 3: 62-66.

Silva, E., S. Batista, P. Viana, P. Antunes, L. Serodio, A.T. Cardoso and M.J. Cerejeira, 2006. Pesticides and nitrates in groundwater from oriziculture areas of the Baixo Sado region (Portugal). Int. J. Environ. Anal. Chem., 86: 955-972.

Stefanidou, M., M. Georgiou, C. Maravelias and A. Koutselinis, 1990. The effects of morphine, cocaine, amphetamine and hashish on the phagocytosis of the protozoon *Tetrahymena pyriformis* strain W. Toxicol. *In vitro*, 4: 779-781.

Stock, C., R.D. Allen and Y. Naitoh, 2001. How external osmolarity affects the activity of the contractile vacuole complex, the cytosolic osmolarity and the water permeability of the plasma membrane in *Paramecium multimicronucleatum*. J. Exp. Biol., 204: 291-304.

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. Toxicol. *In vitro*, 19: 99-105.

Timur, S., S. Onal, U.N. Karabay, F. Sayim and F. Zihniogul, 2002. *In vivo* effects of malathion on glutathione-S-transferase and acetylcholinesterase activities in various tissues of neonatal rats. Turk. J. Zool., 27: 247-252.

Toyama, K., C.H. Bae, J.G. Kang, Y.P. Lim and T. Adachi *et al*., 2003. Production of herbicide-tolerant zoysiagrass by Agrobacterium-mediated transformation. Mol. Cells., 31: 19-27.

Uri, N.D., 1999. The implications of US Government policy on the development and use of biopesticides. Int. J. Environ. Pollut., 11: 117-132.

Van der Oost, R., J. Beyer and N.P.E. Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. Toxicol. Pharmacol., 13: 57-149.

Weckberker, G. and J.G. Cory, 1988. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells *in vitro*. Cancer Lett., 40: 257-264.

Weisse, T., 2006. Freshwater ciliates as ecophysiological model organisms-lessons from Daphnia, major achievements and future perspectives. Arch. Hydrobiol., 167: 371-402.

Yi, X., H. Ding, Y. Lu, H. Liu, M. Zhang and W. Jiang, 2007. Effects of long-term alachlor exposure on hepatic antioxidant defense and detoxifying enzyme activities in crucian carp (*Carassius auratus*). Chemosphere, 68: 1576-1581.

Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev., 74: 139-162.

Yu, M., S.H. Wang, Y.R. Luo, Y.W. Han, X.Y. Li, B.J. Zhang and J.J. Wang, 2009. Effects of the 1-alkyl-3-methylimidazolium bromide ionic liquids on the antioxidant defense system of *Daphnia magna*. Ecotoxicol. Environ. Saf., 72: 1798-1804.