Research Article

Effect of a New Fungicide Combining the Fenamidone and Fosetyl Aluminum on Growth, Respiratory Metabolism and Antioxidant Enzyme Activity of *Paramecium SP.*

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ABSTRACT

The purpose of this work is connected with studying the effects of a systemic fungicide based on fosetyl aluminum and Fenamidone on growth, respiratory metabolism and anti oxidant enzyme activity of *Paramecium sp.* The used concentrations are respectively (0.04, 0.08, 0.16, 0.32 mg/l). The obtained results demonstrate that the cellular growth and respiratory metabolism exhibit a strong inhibition proportionally related to the increasing concentrations of the fungicide. We have also highlighted a strong antioxidant enzyme activity of GST and catalase associated with a net increase in the Biological Anti Oxidant power (BAP's) of the tested cells. In conclusion, the obtained results in the present study revealed that the exposure to the used fungicide induces oxidative stress to *Paramecium sp,* expressed by inhibition of cell growth and respiratory metabolism. Meanwhile stimulation of enzymatic activities; Catalase (CAT) and glutathion-s-transferase (GST) linked to a decrease in glutathion (GSH) rate and an increase in the BAP's rate indicate the triggering of an anti oxidant mechanism in response to the oxidative stress induced by the fungicide.

Keywords: *Paramecium sp*, oxidative stress, Fosetyl-Al, Fenamidone, systemic fungicide, toxicity.

INTRODUCTION

Pollution is an environment's disruption due to the presence of xenobiotic. It affects all the organisms living there, this product and these compounds are released into the environment without any prior treatment necessary for the removal of toxic elements entering into their compositions[1,3]. The massive use of pesticides in the world and particularly in the field of agriculture leads to the contamination of all systems (water, soil, and atmosphere) by these compounds and today present a real danger for populations[4,9]. Among all these xenobiotics, fungicides constitute a large group whose impact on components of environment are still poorly studied. The Verita flash is a systemic and contact fungicide newly marketed, whose action on certain plant diseases especially those designed for fungi (mushrooms)[10].

The fungicide consists of two active substances: the fenamidone and Fosetyl Aluminum[11].

The protozoa are organisms often used as bioindicators of environmental pollution notably that induced by the xenobiotics of chemical nature[12,14].

One of the most used alternative models in this field remains *Paramecium sp* whose behavior and characteristics constitute real parameters revealing the toxicity of studied environmental pollutants. One of the most used alternative models in this field remains *Paramecium sp* whose behavior and characteristics constitute real parameters revealing the toxicity of studied environmental pollutants[15,20].

In this research, we measured some parameters in order to assess the effects of this fungicide on growth, respiratory metabolism and anti oxidant activity of some enzymes causing the triggering of an oxidative stress.

MATERIELS AND METHODS

Cells culture and treatment: the cells of *Paramecium sp* are grown according to the method of 21 incubation is carried out in an oven at 28 °C ± 1 °C and transplanting is done every three days in order to have a population in exponential phase of growth, the cell density is continuously adjusted in order to obtain 10^4 cells/ml.

The treatment by the fungicide is performed after dilution in water in order to prepare 4 concentrations: (0.04, 0.08, 0.16, 0.32 mg/l).

The tests are performed on aliquots of 10 ml of cell culture.

Measurement of Cell Growth

Cell growth of the paramecies' population density is estimated in accordance with[22].

The number of cells is determined by the counting with a microscope (Leica DL 1000) at magnification 40.

Respiratory Metabolism

Cell respiration is followed by a clarck electrode (oxygraph), which allows the oxygen measurement at nanomolar concentrations order.

Paramecium sp is added in the room oxygraph and the fungicide is then supplemented to the different concentrations (0.04, 0.08, 0.16, 0.32 mg/l).

This operation is repeated 3 times and the respiratory kinetics followed during 06 minutes directly on a graph through the computer associated to oxygraph[23].

Assay of Enzymatic Parameters

The Assay of the Reduced Glutathione (GSH)

The assay is carried out according to the method of 24 and the activity of the Glutathione-S-transferase (GST) is determined at λ= 340 nm corresponding to the method of[25].

Catalase Activity (CAT)

The activity of Catalase is directly measured with a spectrophotometer (JENWAY 6505) at a wavelength of 240 nm. According to[26].

Anti Oxidant Biological Potential (BAP's)

Is carried out by using a photometer (FRAS). The amount of used cells for assay is 10 µl.

RESULTS AND DISCUSSION

The effects of the Verita flash on the growth of the *Paramecium sp* population is represented on (Figure 1).

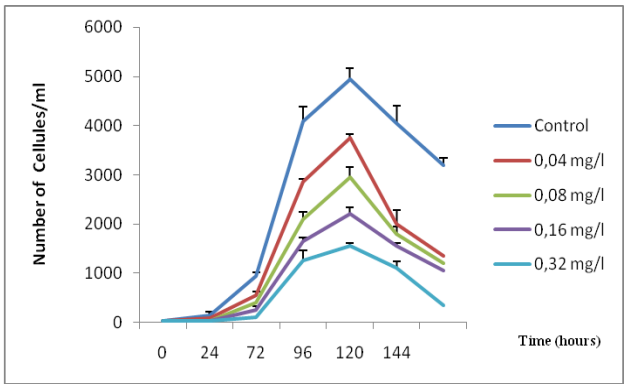

Figure 1: Effect of Verita Flash on the growth of *Paramecium sp.*

We found that the treatment by the fungicide causes an inhibition of density of *Paramecium sp* population compared to the control cells; these is 23% for the weakest concentration, 42% for the concentration 0.08 mg/l, 58% for the concentration 0.16 mg/l and nearly 90% for the highest concentration of 0.32 mg/l.

The obtained inhibition is concentration- dependent[27,28].

These results are in accordance with those reported by[23] who investigated the toxicity of the phosphoramidate and the Chlorfenapyr, as well as the toxicity evaluation three of derivatives amidophosphonates on paramecium[29], and more particularly those of[30] working on *Saccharomyces cerevisaie* which the authors noted an anti proliferation action on the growth curve of cells treated by the nifedipine due to an alteration of the cell cycle and a reduction of the DNA synthesis following a shutdown of factor G_0/G_1 responsible for cellular transition of G_0/G_1 at the phase[28,15] suggested that the treatment by pesticides affects the tested cells by the interference of the used xenobiotics in the operation of the membrane channels calcium that may even cause the cell death.

According to (Figure 02), we found that the respiratory metabolism is heavily used and that even at lower concentration of the fungicide (0.04 mg/l) this due to the inhibition of mitochondrial ATP synthetase which resulted into a significant reduction of the ATP necessary for the cellular metabolic functioning. Similar cases have been reported by[31]. Where the xenobiotic was Nickel. Therefore, Verita Flash appears to act directly on the mitochondrial functioning through ATPase disturbance necessary for proper functioning of the respiratory chain of mitochondria. It is possible that this action that is carried out at the level of ionic exchanges also causes an imbalance of the membrane potential[32].

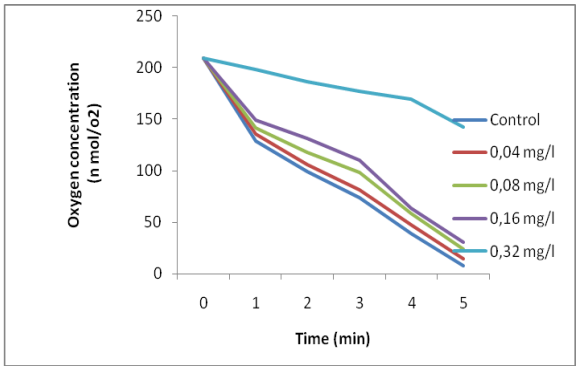

Figure 2: Effect of increasing concentrations of Verita Flash on the respiratory metabolism of *Paramecium sp.*

(Figure 3) illustrates that GSH level when treating cells with fungicide causes a sharp decline in recorded rates especially at high concentration where we recorded a reduction of 75 %.

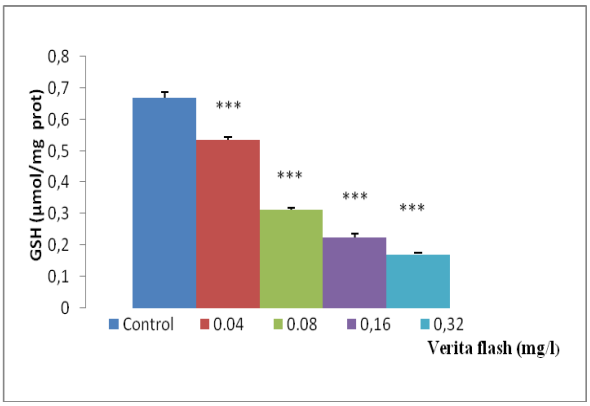

Figure 3: Effect of increasing concentrations of Veria Flash on the evolution of the GSH rate in *Paramecium sp.*

This substrate plays a paramount role in the detoxification of ROS that can be produced by the fungicide.

This result is confirmed by the work of[33]. In parallel the obtained results concerning the GST enzymatic activity are represented in (Figure 4).

These findings highlight an important stimulation of this activity intervening in the conjugation reaction produced GSH. This increase is concentration-dependent and presents a reverse profile to that observed with the GSH. The present result comes to support those reported by[34].

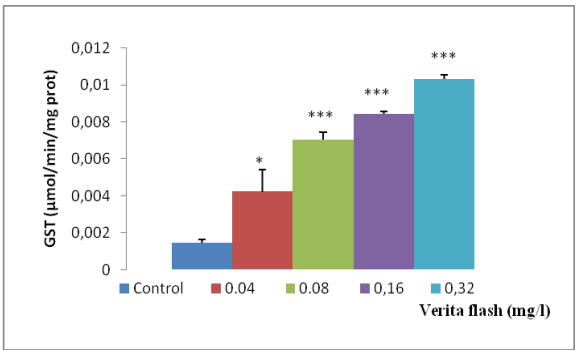

Figure 4: Variation of the GST activity among *Paramecium sp* exposed to increasing concentrations of Verita Flash.

The evolution of the Catalase enzyme activity depending on different concentrations of Verita Flash is represented on (Figure 5). The obtained results show that the Catalase activity is greatly stimulated dose-dependent is particularly at the highest concentration of Verita Flash (0.32 mg/l) where it attains twice that recorded in control cells. The Catalase is an enzyme related to the cell detoxification mechanism responsible for the conversion reaction catalysis of H_2O_2 to water and cellular oxygen[16].

The strong stimulation of Catalase activity observed in our results are an indicator of cellular damage and could reflect an activation of the anti oxidant mechanism necessary for the protection of the treated cells by avoiding as much as possible an accumulation of ROS[30-35]. This important stimulation (P< 0.05) of the Catalase activity compared to control cells expresses a disturbance of the balance Anti/Pro oxidizing (ROS)[36].

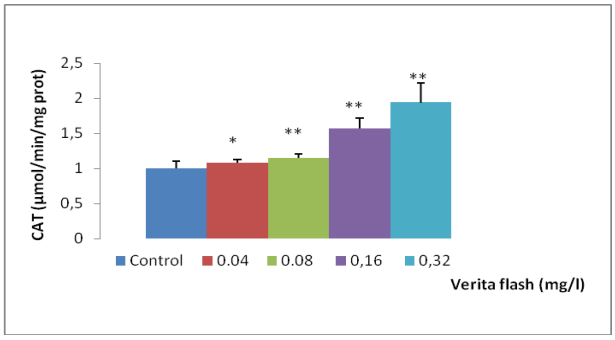

Figure 5: Effect of Verita Flash on the catalase activity of *Paramecium sp.*

Table 1: Effect of increasing concentrations of Verita Flash on the biological antioxidant potential in *Paramecium sp.*

BAP's	Control	0.04 mg/l	0.08 mg/l	0.16 mg/l	0.32 mg/l
µmol/l	6325	8771	8966	9357	10139

Finally, the assay results of Biological Anti Oxidant Potential (BAP's) of the exposed cells to various concentrations of fungicide are summarized in (Table 1). We found that the exposure of *Paramecium sp* to Verita Flash causes an increase of the BAP's which is around 60%. This outcome is important in the sense that it has just confirmed the existence of oxidative stress among the treated cells which is reflected by a synthesis of ROS molecules group. Our results are in agreement with those of[37,30,38].

CONCLUSION

In conclusion, the exposition of *Paramecium sp* cells to different concentrations of the fungicide causes an important toxic effect on all measured physiological and biochemical parameters.

Our results show a clear increased toxicity of the fungicide, which generates an important oxidative stress reflected by a strong disruption of cell growth and respiratory metabolism, accompanied by a synthesis of ROS and therefore the triggering of detoxification system involving a set of anti oxidant enzymes, in synergy with the increase of Biological Anti Oxidant Potential BAP's.

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