EFFECTS OF ATRAZINE, ISOPROTURON, AND DIURON ON GLUTATHIONE METABOLISM OF SACCHAROMYCES CEREVISIAE

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1. Introduction – Under normoxic conditions, living organisms can very efficiently prevent reactive oxygen (ROS) accumulation and attenuate oxidative damages, using various defensive strategies such as those involving the peptide glutathione. However, sudden exposure of eukaryotic cells to xenobiotic as herbicides may cause a redox imbalance of the cell, causing oxidative stress. The yeast *Saccharomyces cerevisiae* is a promising unicellular eukaryotic organism for the toxicological evaluation of xenobiotic as herbicides, because its cellular structure and functional organization have many similarities to those of higher-level organisms. However, the toxicity of phenylureas and triazines herbicides in yeast is still poorly understood. These photosystem II inhibitors herbicides are mainly used in agricultural applications for selective control of germinating grass and broad-leaved weeds in many crops (e.g. cereals). Diuron is also employed for total weed control on non-cultivated areas as maintenance of roads, railways and parks. On the other hand, atrazine (ATZ), diuron (DIU) and isoproturon (IPU) are the most used herbicides in Europe that are often found in contaminated groundwater, surface water, and effluents of wastewater treatment plants. Consequently, the main purpose of this study was to compare the effects of ATZ, DIU and IPU on yeast cell growth and glutathione cycle enzymes.

2. Experimental – *Saccharomyces cerevisiae* UE-ME_3 a wild-type yeast deposited in the collection of laboratory of Enology of University of Évora, at mid-exponential phase, were inoculated in YEPD medium, 2% (w/v) glucose at 28 °C, and shaken 150 rpm during 72 h in presence of ATZ, DIU or IPU (5 μM or 50 μM) and compared with control. Yeasts were harvested by centrifugation at 3000 *g* for 10 min and washed with ultra-pure sterile water. The obtained cells were suspended in 10 mM phosphate buffer pH 7.0, and disrupted by sonication. The post-12000 *g* supernatants were used for glutathione (GSH) and glutathione disulfide (GSSG) determination by fluorescence as well as glutathione reductase (GR) and glutathione peroxidase (GPx) activities by UV-Vis spectrophotometry. The statistical analyses of results were performed by ANOVA I and Duncan test to determine significant differences ($p < 0.01$) between treatments, using SPSS for Windows, version 22, licensed to University of Évora.

3. Results and Discussion – The results show that 50 μM IPU exposure has induced *S. cerevisiae* UE-ME_3 growth, though cell growth has not been affected by 5μM IPU. However, it was observed a significant decrease of yeast growth in cultures containing DIU or ATZ in both levels studied. The buffer capacity mediated by glutathione (GSH/GSSG ratio) has decreased significantly in *S. cerevisiae* grown in presence of both levels of any three herbicides, except in the yeast cells exposed to 50 μM IPU where occurred an unaccountable increase, but positively correlated with the increase of cell growth previously mentioned ($r = 0.786$, $p < 0.01$). On other hand, the results show also a significant decrease of GR activity in the *S. cerevisiae* which grown in the presence of both levels of ATZ, no affecting the same activity in cells exposed to DIU and causing a broad increase in the cells grown in presence of 5 or 50 μM IPU ($p < 0.01$). Interestingly, an identical response profile was determined for GPx activity in the cells exposed to IPU and ATZ, less evident in the case of ATZ. In the case of DIU was detected a decrease in the GPx activity in cells exposed to 5 μM of this herbicide, whereas in the cells exposed to 50 μM DIU it was observed an opposite response. It therefore seems that for higher levels of DIU, the cell machinery seeks to eliminate the oxidative stress induced by the herbicide, consuming peroxides or lipid hydro peroxides generated yet. However the lack of adequate response by GR activity seems to hinder the growth of *S. cerevisiae* UE-ME_3 due a decrease in the intracellular redox power.

4. Conclusions – The chlorine herbicides such as DIU and ATZ inhibited cell growth decreasing the GSH/GSSG ratio and the antioxidant GR activity. Moreover, it seems that 50 μM isoproturon was able to induce cell growth of *S. cerevisiae* UE-ME_3 increasing buffering capacity mediated by glutathione as well as GR and GPx activities. This response may be useful in bioremediation processes of phenylurea isoproturon.