Impact of Pesticides Used in the Culture of the Vine on the Viability of the Yeast Saccharomyces Cerevisiae Wine in Chronological Aging

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ABSTRACT
Pesticides, used in culture, may induce oxidative stress by stimulation of free radicals production, what may result in lipid peroxidation, proteome damage, changes in DNA and RNA structures and disturbance of total antioxidative capacity in organisms’ cells. In disturbances caused by increase synthesis ROS (reactive oxygen species) or lack antioxidative defense that is in oxidative stress it is seen one of all causes of aging process. Chronological aging of baker’s and wine yeast Saccharomyces cerevisiae in liquid stationary culture is used as model of research on the aging process. As a result of aging changes take place in yeast cells which have physiological, genetic, metabolic and morphological character, what cause their death in consequence. Some scientists treat chronological yeast aging as analogy of fibroblasts aging of multicellular organisms, skeletal muscles or nerve cells. The aim of the experiment was to obtain the answer on question connected with toxicity effect two widely available pesticides in shape of trade preparation, used among others in culture of grapevine Miedzian 50 WP (Cu 50WP) and Siarkol Extra 80 WP (S 80WP) on vitality of wine yeast in chronological aging. During research cells of wine yeast Tokay, which are used in production of white wines, and cells of Malaga strain, used in production of white and red wines, were applied. Yeast culture with pesticides supplementation in determined concentrations was conducted through seven days in YPG medium. At that time vitality of yeast cells was determined by the percentage of cells surviving, percentage of dead cells and culture density. Considerable influence on decreasing vitality of yeast cells in the process of aging showed S 80WP, what correlate with the increase of applied concentration in both example of Malaga and Tokay. Obtained results in application of Cu 50WP indicated lower toxicity in culture of both studied strains in comparison to the control. Our studies suggest that studied pesticides may cause the intensity of processes connected with cells aging.

Keywords: Pesticides, wine yeast, chronological aging, toxicity

INTRODUCTION
Pesticides are applied around the world to chemical protection of plants in order to reduce damages from the existence of both vermin and weeds and to increase the harvest of cultivated plants (Wrzosek et al. 2009). Despite that toxic actions of pesticides are directed on exact species there is a possibility, that their remains have disadvantageous health effects on both people and animals (Weiss et al. 2004).

The mechanism of pesticides acting on cellular level, depending on the organism, is very different and not fully elucidated. For example glyphosate (N-phosphonomethylglycine), active component, popularly used in herbicidal preparations, reduces the tissue, which assimilates CO2 in green leaves and as a result is stops the process of photosynthesis. In the last years it was shown that glyphosate does not give in to fast biodegradation, it stays in soil and it may reach to earth water. It was demonstrated that glyphosate with low concentration is detected in blood serum of people (Kwiatkowska et al. 2013). It may induce cancerous changes and affect on hormonal ones. Also it stimulates forming reactive forms of oxygen and it influence on the change in system of potential membrane of mitochondrion, what results in necrosis and apoptosis of cells of different types (Banerjee et al. 2001, Heu et al. 2012). On the other hand, fungicides are directed on disturbances of integrity of cellular membrane or cell wall of fungi on the whole. Despite directed fungical application those chemical means to curing plant diseases have also others mechanisms of acting, including that they stop biosynthesis of sterols and polymerization of microtubules and damages mitochondrial respiratory chain in others than exact organisms. Toxic effect of fungicides may result in instability of cell wall, changes of osmolarity and production of reactive oxygen species (ROS) even within people (Hayes et al. 2014). Therefore pesticides used in culture may induce oxidative stress by stimulation of production of free oxygen radicals (Grosicka-Maciag 2011). It is true that water radicals in physiological concentrations are essential in cell, for example H2O2 acts like signal molecule, but their excess is harmful and it may result in peroxidation of lipids, damage of proteome, changes in DNA and RNA structure and to disturbance of total antioxidative capacity, and even in death of cell (Braconi et al. 2009, Gough and Cotter 2011). Fortunately, organisms are not defenseless against oxidative effect of these oxygen radicals. During course of evolution, with adaptation to oxygen conditions and reactive forms of oxygen, organisms also developed different defense systems against ROS. However, they are not able to eliminate damages caused by those factors completely. The result of it is aging of organisms (Harman 1956, Passos and von Zglinicki 2005, Okasaga 2013). So that we decided to ask whether popular pesticides Cu 50WP and S 80WP have an influence on processes, connected with aging in model of long-term stationary culture of yeast Saccharomyces cerevisiae, which according to many scientists can be concerned as analogy to state of non-proliferating cells of human: fibroblasts, skeletal muscles and nerve cells (Longo et al. 1996, Chen and Runge 2009, Balazsi 2010). Chronological aging of yeast is commonly used in studying of aging processes (Grey et al. 2004, Longo et al. 2012) (figure1).
RESULTS

In presented study on the pesticide influences on process of yeast aging culture we checked the aging indicators, such as density culture, the viability of cells and the number of dead cells. Results are shown in figures 2 - 7.

During the first day of culture significant drop of culture density of Malaga in supplementation of Cu 50WP and S 80WP was observed in comparison to control. Especially addition S 80WP decreased density about 30% in comparison to control. Also toxic result of S 80WP influenced Tokay cells. Whereas Cu 50WP indicated differentiated effect on level of culture density of Tokay and with concentration of 2 mM significant increase of culture density was noted in comparison to control (figure 2).

After seven days of culture pesticides Cu 50WP and S 80WP decreased culture density in both studied strains significantly in comparison to control. Especially S 80WP turned out to be toxic for both Malaga and Tokay (figure 3).

All experiments were carried out in three independent repeats.

**Statistical analysis**

All data are expressed as the mean ± standard deviation (SD). The Mann–Whitney U test was used for analyzing the results. We used the following software for statistical analyses: STATISTICA – StatSoft Polska sp. z o. o.(version 10.0 for Windows), p<0.05.)
After 7 day in culture there was twice decrease of survival due to Cu 50WP in Malaga strain (figure 5). Observed decrease was dosage independent. In Tokay strain decrease of survival also was observed, however it was dosage dependent and 0.5 mM Cu 50WP induced only 15% downregulation, 2 mM and 5 mM Cu 50WP caused 40% and almost 100% decrease, respectively. S 80WP in 7 days time point severely reduced the survival level in both yeast strands and in all tested concentrations.

On the first day of culture it was observed increase of percentage of dead cells in culture subjected to pesticides effect in comparison to monitoring cultures in both studied yeast strains. Cultures of Malaga yeast indicated significant increase of percentage of dead cells, caused by pesticides Cu with concentration of 2 mM in all applied concentrations (0.5 mM, 2 mM, 5 mM) of S 80WP. Cultures of Tokay yeast indicated significantly, even to 50%, higher percentage of dead cells under the influence of pesticide Cu 50WP (2 mM and 5 mM) and up to 60% under the influence of pesticide S 80WP in all applied concentrations in comparison to control (figure 6).

The addition of pesticides Cu 50WP and S 80WP in proposed concentrations of culture of wine yeast Malaga andTokaj significantly decreased cells vitality culture. It was observed that toxic influence of Miedzian was a little lower in cultures of both studied strains in comparison to S 80WP with reference to control. That result can explain that effect of sulphur is bipolar. Sulphur, as active substance S 80WP, gets in to fungi cell due to its solubility in fats, disintegrates cellular membrane and causes water flow from the cell, which results in death of fungi (Ciesielska et al. 2011). Also sulphur indicated effect on level of respiratory system (cytochrome b), which comes in place of oxygen as acceptor of electrons. As a result of sulphur reduction hydrogen sulphide comes into being, which prevents ATP form coming into existence and causes significant loss of energy on cellular level (Ciesielska et al. 2011). Wheras copper contained in Cu 50WP effects on superficial mainly. Copper ions gets in to semipermeable membrane and chitinous wall of fungi scores, driving out ions of hydrogen, causing water flow from the cell, which results in death of fungi (Ciesielska et al. 2011).
calcium and magnesium. Then inactivation of structural and enzymatic proteins of cellular membrane takes place (Ciesielska et al. 2011). Copper compounds in shape of oxychlordime also participate in inhibition of energetic processes and respiratory enzymes in spray fungicides and antifungal proteins. Hypothesis can be taken into consideration that both pesticides Cu 50WP and S 80WP may also cause to appearance of free spores in yeast cells. This may result in increased chronological aging of yeast cells (Longo et al. 2012). As a result of accumulation of oxidative damages and high level of superoxide anion yeast cells lose ability of budding in chronological aging (Demir and Koc 2010). Whereas increase of surviving yeast cells in both studied strains on the seventh day of stationary culture in presence of Miedzian in comparison to the first day of culture may prove about cells adaptation to oxidative stress known as mitohormesis (Kharade et al. 2005, Piper et al. 2006, Ristow and Zarse 2010). In the presence of sulphur in Malaga culture shown increased sensitivity on that pesticide. Our studies suggest, that studied pesticides may cause to increase processes, connected with cells aging. In the next stage of research we are planning to check the level of total antioxidative ability and formation of superoxide anion in those aging cultures.

REFERENCES


CIESIELSKA, J., MALUSA, E., SAS PASZT, L. 2011. Środki ochrony roślin stosowane w rolnictwie ekologicznym. Skiermiewice, Drukarnia PPHU “Graf-Sad” SC


