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*Original Research*

# The Influence of Selected Pyrethroid on the Growth and Number of ρ-Mutants in Saccharomyces Cerevisiae Yeast

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**Abstract**

The main purpose of this study was to examine the influence of pyrethroids, such as deltamethrin, cypermethrin and bifenthrin on the growth and the number of ρ-mutants in the cells of *Saccharomyces cerevisiae* yeast. SP-4 Mat alpha leu1 arg4 yeast strain was used as a standard strain for experiments. The cells were grown on a standard YPG liquid medium, under aerobic conditions until they reached the logarithmic or stationary phase of growth. Different concentrations of pyrethroid were added to the medium and the cells were incubated for 2 h. The survival rate of the cells was determined by diluting the cells and plating them on YPG Agar plates. The number of ρ-mutants was determined by examining the number of cells that hadn't grown on YPG medium from among all surviving cells. As far as the investigated forms of pyrethroids are concerned, deltamethrin was the most toxic to yeast cells. It was also observed that low amounts of pyrethroid caused a greater destruction of cells at the logarithmic than at the stationary stage of growth. The influence of pyrethroid on the frequency of mutation of mitochondrial DNA of yeast cells was also studied. It was observed that after incubation with pyrethroid addition the frequency of ρ-mutation increased, especially at the logarithmic stage of growth.

**Keywords:** yeast, pyrethroid, ρ-mutant.

## Introduction

Pyrethroids are esters of primary or secondary alcohols containing at least one double bond and chrysanthemic acid (2.2-dimethyl-3-(2-methylpropenyl)-cyclopropanecarboxylic acid), or halogen analogues of this acid. These pesticides are neurotoxins which inhibit the transfer of nerve impulses by blocking nicotinic acetylocholine receptors and receptors for gamma-aminobutyric acid in the nerve cell membranes of insects [1]. Such compounds as deltamethrin, cypermethrin and bifenthrin are commonly used in agriculture, veterinary medicine and households, as they do not accumulate in the environment and are not very toxic to mammals and birds. These compounds are also quickly metabolized and eliminated from the bodies of mammals [2]. However, the widespread use of pyrethroids raises concerns about their non-specific effects on the environment and on human beings. Exposure to pesticides increases the risk of neurodegenerative diseases such as Parkinson's disease in humans [3]. The non-specific effect of pyrethroids on cells may concern various metabolic processes. It has been determined that deltamethrin induces oxidant stress in the kidneys and liver of the *Channa punctatus* fish [4] and in the organs of rats [5-7].

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Studies on the mutagenic influence of pyrethroids have been conducted on various plants, bacteria, insects, mammals and human cell lines. It should be emphasized that most of these experiments concerned the effect of pyrethroids on the nuclear genome. The *Saccharomyces cerevisiae* yeast is a recognized model in studies on the toxicity of various chemical compounds which affect mitochondrial DNA [8]. These compounds cause a ρ-mutation which manifests itself by the inability of the cells to grow on non-fermentable carbon sources [9]. ρ-mutants are characterized by the deletion of mitochondrial DNA (mDNA) and no mitochondrial protein synthesis takes place in their mitochondria. ρ-mutants are also called petites, because they form smaller colonies than the wild yeast strain growth on the media containing a limited amount of glucose. ρ-mutants grown on this medium are characterized by small colonies and a whitish color, whereas non-mutant cells (ρ+) form large, beige-colored colonies. The spontaneous frequency of this mutation is rather high; about 2% of the yeast cell population consists of ρ-mutants. The loss of ability to perform oxidative phosphorylation by yeast cells is not lethal, contrary to the cells of higher eukaryotes [8]. Studying mitochondrial DNA mutation in higher eukaryotes is costly, and using *S. cerevisiae* yeast makes it possible to determine the sensitivity of mDNA to xenobiotics relatively easily. The subject of this study was the influence of pyrethroids such as deltamethrin, cypermethrin and bifenthrin on the survival rate on the cells of *Saccharomyces cerevisiae* yeast and the frequency of ρ-mutation in their mitochondrial DNA at the logarithmic and stationary stages of culture growth.

## Experimental Procedures

The studies were conducted on a wild strain of the SP4 – Mat alpha leu1 arg4 yeast genotype [10]. The yeast cells were grown on a liquid YPG medium containing 1% yeast extract, 1% pepton and 2% glucose. 2% agar was added to the solid YPG medium.

The pyrethroids were purchased at the Organic Industry Institute in Poland:
bifenthrin – (2-methyl-1.1-biphenyl-3-y1)-methyl-3-(2-chloro-3.3.3-trifluoro-1-propenyl)-2.2-dimethyl cyclopropane-carboxylate,
deltamethrin – (S)-alpha-cyano-3-phenoxybenzyl (1R)-cis-3-(2.2-dibromovinyl)-2.2-dimethylcyclopropane-carboxylate,
cypermethrin – (R,S)-alpha-cyano-3-phenoxybenzyl(1RS)-cis,trans-3-(2.2-dichlorovinyl)-2.2-dimethylcyclopropane-carboxylate.

In the logarithmic or stationary stages of growth the yeast cultures were incubated for two hours with additions of various concentrations of pyrethroids and their survival rate was determined by observing the ability of the cells to form colonies on a solid YPG medium. Results of our experiments have shown that a change in conditions, *e.g.* a two–hour incubation of the cells in a buffered solution may bring about a stress reaction in yeast cells; consequently, pyrethroids were added directly to the yeast culture.

The number of ρ-mutants was determined by plating a suspension with a known number of yeast cells onto solid media with the addition of ethanol or glucose and then comparing the number of colonies which grew on these media. Finally, the percentage of colonies which did not grow on the YPE medium was calculated [8].

The obtained results constitute a mean of three or more independently conducted experiments. The mean and standard deviation were calculated using an Excel 7.0 spreadsheet.

## Discusion of Results

The aim of the first stage of the experiments was to study the influence of various concentrations of the pyre-

Table 1. Effect of pyrethroids on survival rate and on the number ρ-mutants in logarithmic yeast cells.

| Pyrethroid concentration $\mu g \cdot cm^{-3}$ | Bifenthrin | | Cypermethrin | | Deltamethrin | |
|---|---|---|---|---|---|---|
| | Survivors% | ρ- mutants% | Survivors% | ρ- mutants% | Survivors% | ρ- mutants% |
| 0 | 100 ± 2 | 0.5± 0.2 | 100± 2 | 0.5± 0.2 | 100± 2 | 0.5± 0.2 |
| 5 | 96±3 | 3±2.8 | 97±4.1 | 3±2.3 | 92±4.6 | 14±6.1 |
| 10 | 96±3.4 | 12±3.7 | 88±3.5 | 25±2.6 | 81±5.7 | 34±4.5 |
| 15 | 92±2.6 | 31±4.6 | 83±4.3 | 21±5.3 | 72±4.2 | 30±6.3 |
| 20 | 81±5.1 | 38±4.2 | 65±4.3 | 34±5.6 | 43±4.5 | 38±5.2 |
| 25 | 67±3.6 | 36±6.7 | 58±4.5 | 34±6.4 | 26±3.6 | 40±6.7 |
| 30 | 43±4.5 | 32±5.9 | 47±2.7 | 36±5.1 | 11±4.3 | 25±6.2 |
| 40 | 15±2.9 | 35±8.3 | 23±5.1 | 43±4.2 | 0 | 0 |
| 50 | 4±4.1 | 40±6.7 | 15±3.5 | 42±3.6 | 0 | 0 |
| 60 | 0 | 0 | 0 | 0 | 0 | 0 |

throids in question on the survival rate of yeast cells (Tables 1 and 2). It was found that the cells were more sensitive to these pesticides in the logarithmic stage than in the stationary stage. Loss of viability of all the yeast cells at the logarithmic stage was achieved by applying deltamethrin at a concentration of 40μg·cm$^{-3}$, and cypermethrin and bifenthrin at a concentration of 60 μg·cm$^{-3}$ (Table 1), loss of viability of the cells at the stationary stage was achieved by applying deltamethrin at a concentration of 140 μg·cm$^{-3}$ and bifenthrin and cypermethrin at a concentration of 200 μg·cm$^{-3}$ (Table 2). From among the pyrethroids under study, the toxicity of deltamethrin was the highest; 50% mortality rate of yeast cells was noted at the logarithmic stage after the application of deltamethrin at a concentration of 20μg·cm$^{-3}$, and after the application of bifenthrin and cypermethrin at a concentration of 40μg·cm$^{-3}$. Similar differences were noted in the stationary phase, where mortality rates reached over 50% for deltamethrin at a concentration of 80μg·cm$^{-3}$, and for cypermethrin and bifenthrin at the concentration of 140 μg·cm$^{-3.}$

In the study of the effect of pyrethroids on the mitochondrial DNA of yeast cells, the criterion for mutation was the percentage of ρ-mutants in the surviving population of cells. The ρ-mutation is manifested phenotypically as an inability of cells to grow on a non-fermentable carbon source. It was determined that after incubation with the pyrethroids under study there was an increase in the frequency of ρ-mutation among surviving cells, especially in the logarithmic stage of growth (Tables 1, 2).

## Discussion

*Saccharomyces cerevisiae* yeast is a recognized model organism used in studies concerning the toxicity of various xenobiotics, including pesticides. Result obtained from the conducted experiments make it possible to state that the examined pyrethroids are toxic to yeast cells. Deltamethrin had the most destructive influence on the cells; it lowered their survival rate even when its concentration was as low as 5μg·cm$^{-3}$. Comparison of the survival rates of yeast cells incubated with the addition of pyrethroids at different stages of yeast growth showed that yeast cells at their stationary stage of growth tolerate higher concentration of pyrethroids than at their logarythmic stage of growth. At the stationary stage of growth yeast cells are more resistant to the effects of toxic factors, presumably because the activity of antioxidant enzymes is greater than in the logarithmic phase [11]. Pyrethroids can affect cells by generating oxidant stress. This process is accompanied by a response of the antioxidant system of the cells, manifested by a change in the activity of the antioxidant enzymes, such as supraoxide dismutase and catalase, and by changes in the concentration of antioxidants [5-7].

Pyrethroids are more toxic compounds for yeast than, for example, paraquat – a compound which generates free radicals. Total destruction of yeast cells took place when the concentrations of paraquat was 2mg·cm$^{-3}$ [12]. In studying the effect of Fastac 10EC preparation showed that 50% of the yeast population died when the concentration of alfamethrin was 6.5μg·cm$^{-3}$ [13]; for human lymphocytes the cytotoxic concentration of pyrethroids is 3-6μg·cm$^{-3}$ [14]. Cypermethrin is toxic for rat erythrocytes at concentrations of 200ng·cm$^{-3}$ and higher [15].

The study also showed that among the surviving yeast cultures there are ρ-mutants. Loss of mitochondrial functions protects yeast cells from the toxic effect of reactive forms of oxygen. ρ-mutants are more resistant to chemical compounds which generate reactive forms of oxygen than wild strains [8].

Mutagenic properties of pyrethroids have been confirmed by studying the effect of pyrethroids on human

Table 2. Effect of pyrethroids on survival rates and on number ρ- mutants in stationary yeast cells.

| Pyrethroid concentration μg·cm$^{-3}$ | Bifenthrin | | Cypermethrin | | Deltamethrin | |
|---|---|---|---|---|---|---|
| | Survivors% | ρ–mutants % | Survivors% | ρ–mutants % | Survivors% | ρ–mutants % |
| 0 | 100±1.9 | 1±0.8 | 100±1.9 | 1±0.8 | 100±1.9 | 1±1.9 |
| 20 | 100±2.7 | 3±2.4 | 100±1.3 | 6±2.2 | 91±5.2 | 12±4 |
| 40 | 100±2.2 | 5±3.9 | 100±1.5 | 9±2.4 | 88±4.1 | 16±3.2 |
| 60 | 97±4.1 | 15±4.6 | 93±3.7 | 14±2 | 68±3.2 | 28±4.8 |
| 80 | 92±3.5 | 17±2.8 | 86±2.4 | 19±3.3 | 45±3.5 | 20±6.5 |
| 100 | 80±4 | 27±3.7 | 82±3.8 | 19±2.4 | 29±3.5 | 31±4.2 |
| 120 | 73±6.5 | 35±4.1 | 56±3.6 | 23±3.7 | 9±3 | 35±3.7 |
| 140 | 48±3 | 30±3.6 | 41±2.2 | 26±4.1 | 0 | 0 |
| 160 | 22±5.7 | 28±5.9 | 15±4.1 | 24±3.6 | 0 | 0 |
| 180 | 10±4.8 | 21±2.8 | 6±3.9 | 22±3.1 | 0 | 0 |
| 200 | 0 | 0 | 0 | 0 | 0 | 0 |

lymphocytes, ovary cells in hamsters and on the meristematic tissue of *Vicia faba* and *Allium cepa* [14]. The mutagenic effect of supercypermethrin on four different organisms was compared, and it was determined that it is not mutagenic for the strain *Salmonella typhimurium*; it induces point mutations in *Saccharomyces cerevisiae* in the locus for isoleucine and gene conversion in the locus for tryptophan. The frequency of chromosome aberrations was slightly increased in *Vicia faba*; and no genotoxic effect was found in *Drosophila melanogaster* [16]. The results of studies on the mutagenic effects of pyrethroids are ambiguous, the difficulty in assessing the genotoxic effects of pyrethroids can be connected with the metabolic activity of the organisms studied [14].

Pollution of the environment with pyrethroid pesticides is becoming a serious problem. Research conducted in Germany has shown that the products of pyrethroid metabolism appear in the urine of people who have not had a direct contact with pyrethroids [17]. Particularly worrisome is the fact that, in the authors' opinion, daily diet is a basic source of pesticide contamination in the human body. Long-term exposure to the low dosages of pyrethroids present in the household or at workplaces (in rugs, wallpaper or fabrics) causes chronic illnesses [18]. Exposure to pesticides increases the frequency of cancers in humans. Insecticides, herbicides and fungicides can cause cancers of the blood, prostate, pancreas and liver [19].

In humans the number of mitochondrial DNA mutations increases with age and can cause many diseases and disorders associated with old age [20]. It has been determined that chemical compounds which induce ρ-mutations in yeast can be carcinogenic for mammal cells, and a process comparable to the early stages of mutagenesis in yeast cells takes place in human mitochondria during the onset of degenerative diseases or those associated with age. Exposure to factors causing ρ-mutations may increase the frequency of degenerative diseases in humans [9].

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