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Anna KRZEPIŁKO[1] and Agata ŚWIĘCIŁO

DO ANTIOXIDANTS COUNTERACT THE TOXIC EFFECTS OF PYRETHROIDS ON *Saccharomyces cerevisiae* YEAST?

CZY ANTYOKSYDANTY PRZECIWDZIAŁAJĄ SKUTKOM TOKSYCZNEGO ODDZIAŁYWANIA PYRETROIDÓW NA KOMÓRKI DROŻDŻY *Saccharomyces cerevisiae*?

Abstract: Pyrethroids are synthetic 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 it. These compounds have been used as insecticides. Their mechanism of toxic action on insects consists in inhibiting the activity of ion channels in nerve cells. According to data from the literature, generation of reactive forms of oxygen may be the mechanism of numerous non-specific reactions induced by these pesticides in various organisms. The aim of this study was to determine whether supplementing media with antioxidants protects *Saccharomyces cerevisiae* yeast cells from loss of viability caused by incubation with pyrethroids. The yeast cells were incubated for 2 h with selected pyrethroids and then plated on solid medium containing various antioxidants. The survival rates of yeast cells grown on control media and enriched media were compared. The antioxidants applied were not found to protect the yeast cells from the toxicity of the pyrethroids.

Keywords: pyrethroids, yeast, antioxidants

Pyrethroids are synthetic 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 [1]. The literature dealing with the biological activity, mechanisms of action, and toxicity of pyrethroids is very extensive. Authors of many studies have stressed the acute toxicity of pyrethroids for insects, fish, and other aquatic organisms [2–4]. These compounds inhibit ion channel (sodium channel) function in the nerve cell membranes of insects, leading to their death [5]. Pyrethroids, like other xenobiotics, can affect the functioning of cells of all organisms and lead to potentially dangerous biochemical consequences, such as changes in the activity of enzymes, including those responsible for antioxidant protection of

[1] Department of Agricultural Sciences in Zamość, University of Life Science in Lublin, Szczebrzeska 102, 22–400 Zamość, Poland, phone 84 67 727 724, email: akrzepilko@wnr.edu.pl

cells; atypical metabolic processes; acceleration of ageing processes; and endocrine system disturbances.

According to data from the literature, the common mechanism of these non-specific reactions of organisms to pyrethroids may be free radical generation [6]. When the toxic effects of pyrethroids on yeast cells were studied, changes characteristic of oxidative stress were observed, such as changes in catalase and superoxide dismutase activity, a decrease in reduced glutathione, and decreased concentration of thiol groups and total antioxidant capacity [7–9].

Physiological processes involved in cellular oxygen metabolism lead to generation of reactive oxygen species – (ROS). A balance between production and elimination of ROS is maintained thanks to antioxidant enzymes and antioxidants. Disruption of this homeostasis is manifested by increasing concentration of free radicals, and leads to oxidative stress [10]. Many different environmental factors are responsible for free radical generation, including pesticides.

A group of compounds with antioxidant functions, having very diverse chemical structure, occurs in the cells of all organisms. The task of these molecules is to react with free radicals and inhibit free radical reactions in the early stages of their propagation. Free radicals can react nonspecifically with antioxidants such as glutathione, cysteine, vitamins A, C, and E, uric acid, taurine, metallothioneine, and plant polyphenols. Vitamin E, α-lipoic and coenzyme Q are the most important antioxidants protecting the lipids of biological membranes from free radicals [10]. Ascorbic acid and glutathione prevent spreading of free radical reactions in the hydrophilic environment [11]. The literature provides numerous examples of the synergistic effects of hydrophobic and hydrophilic antioxidants (vitamins E and C, glutathione and vitamin A) [12].

Yeast cells are incapable of synthesizing tocopherols and ascorbic acid, but they easily take up these components from medium and build them into their cellular structures. *S. cerevisiae* yeast produce erythroascorbic acid, but its concentration is considerably lower than ascorbic acid concentration in the cells of other eukaryotic organisms [13].

The aim of this study was to test whether supplementing medium with antioxidants would protect *S. cerevisiae* yeast cells from death caused by incubation with pyrethroids. The study used antioxidants that act within the hydrophilic environment (ascorbic acid) and the hydrophobic environment (alpha-tocopherol) of cellular organelles, as well as their derivatives ascorbic acid 6-palmitate (which acts within the cell membrane environment) and alpha-tocopherol acid succinate (which, unlike tocopherol, acts within the hydrophilic environment of the cell).

Material and methods

Yeast strain: a wild-type strain of the yeast SP-4 phenotype Mat α leu1 arg4 [14]. Culture conditions: The yeast were grown in liquid YPG medium in standard conditions to the late logarithmic phase. The yeast cells were incubated for two hours with the following pyrethroids: cypermethrin, fenvalerate, tetramethrin, and permethrin. The pyrethroid solutions were diluted so that their final concentration in the incubation

mixture was 50 and 100 μg · cm^{-3}. Following incubation with pyrethroids the yeast cell suspension was diluted to a density of about 1–5 × 10^3 cells · cm^{-3} and plated on Petri dishes with solid YPG medium + 2 % agar supplemented with antioxidants in concentrations that do not reduce the survival rate of yeast cells. The antioxidant solutions were rubbed into the solidified medium directly before plating. After plating, the Petri dishes were incubated for two days at a temperature of 28 °C and then the number of colonies was counted. The survival rates of the yeast cells were determined as percentages. 100 % was taken to be the number of colonies obtained in the control sample.

Results

The aim of the first part of the experiment was to determine the effect of selected antioxidants on the survival rate of yeast cells (Table 1).

Table 1

Survival rate [%] of wild type SP4 (*wt*) yeast cells in the presence of selected antioxidants

Type and final concentration [mM] of antioxidants	Survival rate [%] of wild type SP4 (*wt*) yeast cells
control	100
(±)-α-Tocopherol	
0.015	105.3
0.03	91.5
0.045	94.5
0.06	84.8
0.09	85.9
(+)-α-Tocopherol acid succinate	
0.006	104.8
0.012	97.8
0.025	92.5
0.036	98.4
0.125	90.7
0.25	94.8
0.375	86.1
Ascorbic acid 6-palmitate	
0.016	102.5
0.03	86.8
0.05	79.8
0.16	78.5
0.32	82.0
0.48	80.4
Ascorbic acid	
40	94

The lower survival rate of the yeast cells following application of higher doses of tocopherol, tocopherol succinate and ascorbic acid 6-palmitate indicates that these

Table 2

Survival rate [%] of wild type SP4 (*wt*) yeast cells in the late logarithmic phase of growth in the presence of selected antioxidants after previous incubation with cypermethrin or fenvalerate

Type and final concentration of antioxidants [mM]	Type and final concentration of pyrethroids [μg · cm^3]							
	cypermethrin		fenvalerate		tetramethrin		permethrin	
	50	100	50	100	50	100	50	100
Survival rate after incubation with pyrethroid	78.5	47.7	100	25.3	76.2	41.3	87.4	25.2
(±)-Alpha-tocopherol 0.015	95.2	37.8	103.2	13.25	69.5	51.3	83.8	32.9
α-Tocopherol acid succinate 0.006	84.7	23.9	102.7	10.95	89.7	34.9	79.9	30.2
Ascorbic acid 6-palmitate 0.016	78.3	33.1	119	24.1	77.9	37.7	72.7	22.6
Ascorbic acid 40	85.2	36.4	97.1	29.6	75.2	40.8	86.4	23.9

antioxidants may exhibit pro-oxidant activity. Ascorbic acid concentration was determined in earlier experiments [15]. Yeast are capable of growing in the presence of high doses of vitamin C. Exceeding a dose of 80 mM lowers the survival rate of cells in air. Pyrethroids lower the survival rate of yeast cells in the late logarithmic phase of growth. When a 50 $\mu g \cdot cm^3$ dose was applied, cypermethrin was found to be the most toxic of the pyrethroids, followed by tetramethrin and permethrin, while fenvalerate did not reduce the cell's ability to form colonies. The higher, 100 $\mu g \cdot cm^3$ dose of pyrethroids was more toxic; permethrin and fenvalerate caused a severe reduction in survival rate.

To test whether supplementation with antioxidants protects against the toxic activity of pyrethroids, yeast cells were incubated with cypermethrin, fenvalerate, tetramethrin, or permethrin, and then plated on media containing antioxidants. However, the antioxidants were not found to significantly influence the survival rate of the yeast cells. A slight increase in survival rate in the sample with alpha-tocopherol was found only in the case of the lower dose of cypermethrin, tetramethrin and permethrin. Following incubation with 50 $\mu g \cdot cm^3$ of cypermethrin, and subsequent plating of cells on medium enriched with alpha-tocopherol, survival rate increased about 16 %. For tetramethrin and permethrin, the survival rate increased 13 % and 10 %, respectively.

Discussion

Pyrethroids are divided into two types: type I lacks a cyano group (*cis* and *trans* permethrin), while type II contains a cyano group (cypermethrin, fenvalerate). Surrales postulates [16] that the toxicity of pyrethroids for mammal cells may be modulated by the organism's metabolic activity. Biodegration products of pyrethroids do not exhibit insecticidal activity, but are more toxic than the parent pyrethroid for mammals and aquatic organisms. The mechanism of action of many pesticides involves inhibition of mitochondrial complex I activity [17]. During metabolism of cypermethrin, cyanohydrin is formed, from which thiocyanate anions are generated [1]. Various symptoms of poisoning with type I and II pyrethroids have been noted in mammals. Rats given type I pyrethroids exhibit symptoms of poisoning known as the T-syndrome – aggressive behaviour, ataxia, muscle tremors, and convulsions, while type II pyrethroids induce the CS-syndrome, manifested by hypersensitivity, epileptic seizures, chorea, and ptyalism, as well as a burrowing reflex. Type II pyrethroids attack the central nervous system, while type I pyrethroids attack the peripheral nerves [18]. Cyanides and thiocyanates are inhibitors of mitochondrial respiratory enzymes. This toxicity mechanism of pyrethroids has marginal significance in yeast. In the case of yeast cells, inhibition of the respiratory chain does not lead to cell death, because yeast are facultative anaerobes. Nevertheless, following incubation with pyrethroids, an increase in the number of rho-mutants, which are incapable of growing on non-fermentable carbon sources, has been observed among the surviving cells [8].

Induction of oxidative stress is one of the main mechanisms of action of many pesticides. Damage to membrane lipids, protein and DNA is the endpoint biomarker of the oxidative stress-inducing effects of pesticides [19]. Data from the literature provide

numerous examples demonstrating that pyrethroids cause changes in the activity of antioxidant enzymes and in the concentration of antioxidants in the cells of various organisms.

Changes in parameters characteristic of oxidative stress have also been observed in the case of yeast cells. Our previous experiments have shown that mutants lacking the main antioxidant enzyme, superoxide dismutase, are more sensitive to pyrethroids. We have also published the results of an experiment confirming that pyrethroids cause a disturbance of redox potential and reduce concentration of GSH and thiol groups [7, 8]. Decreased total antioxidant capacity in yeast cell extracts suggests that the antioxidant system is involved in the detoxification of pyrethroids [20].

Under certain conditions, antioxidants protect cells from uncontrolled free radical reactions. The literature provides many examples supporting the thesis that antioxidants are capable of inhibiting or mitigating a disease process involving free radicals [21, 22].

Mutant *sod 1* yeast lacking the main antioxidant enzyme, superoxide dismutase Cu Zn SOD, is hypersensitive to the effects of pro-oxidant factors, and unlike the wild type strain, is not capable of growing in an oxygen atmosphere. Supplementing the medium with ascorbic acid enables cells of the *sod 1* mutant to grow in an oxygen atmosphere [15]. This example confirms that the commonly used antioxidant ascorbic acid can protect cells of the *sod 1* yeast mutant from free radicals.

Ascorbate in the presence of transition metal ions can oxidize and stimulate free radical reactions. One of the most dangerous potential reactions is reduction of iron ions to Fe^{2+} (the Fenton reaction), which can enhance generation of hydroxyl radicals. The oxidized form of vitamin C, dehydroascorbate, can damage erythrocyte membranes and affect various metabolic reactions [10]. The antioxidant mechanism of alpha-tocopherol does not involve reaction with oxygen, but rather interception of the free radical chain reaction, which is perpetuated not by oxygen but by fatty acids. Alpha-tocopherol reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation, and intercepts the chain reaction [23]. The tocopherol radical generated in this reaction can react with other antioxidants, such as glutathione, and be regenerated into tocopherol. A reaction between glutathione and tocopherol is catalyzed by an enzyme that reduces the tocopherol radical [12].

The results of an experiment using antioxidants and pyrethroids was published by Kale et al [24], who found that vitamin E protects rat tissues from oxidative stress that has been increased by pyrethroids. Changes characteristic of oxidative stress were less severe in animals that had been given vitamin E and then pyrethroids than in animals treated only with pyrethroids. These changes included a high level of lipid peroxidation products and a higher level of activity of superoxide dismutase, catalase and glutathione transferase than is found under physiological conditions. Vitamin E only partially protected acetylcholinesterase from inhibition caused by pyrethroids. A histological picture of liver tissues has also confirmed the protective role of vitamin E. Cypermethrin and fenvalerate induced necrotic changes in the liver, but administration of vitamin E protected the physiological structure of hepatocytes and counteracted the destruction of cells exposed to pyrethroids.

Vitamin E protects cell membranes against lipid peroxidation, which is one of the frequently observed symptoms of pyrethroid toxicity [25]. The mechanism of action of pyrethroids on cell membranes depends on the type of molecular structure. Type I pyrethroids, which have no cyano group (eg permethrin), penetrate biological membranes and affect the activity of intracellular enzymes [18]. Type II pyrethroids, which contain a cyano group, have a more hydrophilic molecular structure and it is more difficult for them to overcome the barrier of the cell membrane. For this reason they have a stronger effect on the cell membrane structure and damage it. In the case of yeast cells, the lipid peroxidation process has only marginal significance, as about 80 % of all lipids in the plasma membranes of yeast are monounsaturated and saturated fatty acids [26]. Nevertheless, pyrethroids cause yeast cell membranes to lose integrity, which was confirmed by staining conducted in previous studies. Pyrethroids can affect the functioning of ion channels in membranes, which disorganizes transmembrane transport. Gabianelli postulates [4] that pyrethroids can act on cells in two ways: by inducing oxidative stress in the cytoplasm and, due to their hydrophobicity, by accumulating in biological membranes.

A new way of looking at the toxicity of pesticides, including pyrethroids, is postulated by Kaseras and Mantion [26]. Many studies on the effects of pyrethroids and other pesticides (organochlorines, organophosphate pesticides) have shown that they affect intracellular ion balance, particularly of calcium. Even slight changes in intracellular Ca^{2+} concentration induced by these pesticides affect the activity of constitiutive nitric(II) oxide synthetase. One of the products of the reaction catalyzed by this enzyme is nitric(II) oxide. NO is rapidly oxidized by non-enzymatic reactions to form NO_3 (nitrate). NO also reacts with glutathione to form nitrosothiol or with heme to yield heme-NO [27]. Depending on conditions, nitric(II) oxide can exhibit strong pro-oxidant activity and increase oxidative stress. It can also exhibit cytotoxic activity. Pesticides induce a number of non-specific reactions by disrupting nitric(II) oxide metabolism. This short-lived molecule can affect many metabolic processes; it acts as a free radical, a second messenger, a neurotransmitter, or a hormone. A disturbance of calcium homeostasis causes a reduction in constitutive NO production, leading to cellular excitability and impairment of immunity (chemotaxis) and affecting signal transmission pathways, which precludes a cellular response to negative environmental factors.

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CZY ANTYOKSYDANTY PRZECIWDZIAŁAJĄ SKUTKOM TOKSYCZNEGO ODDZIAŁYWANIA PYRETROIDÓW NA KOMÓRKI DROŻDŻY *Saccharomyces cerevisiae*?

Wydział Nauk Rolniczych w Zamościu
Uniwersytet Przyrodniczy w Lublinie

Abstrakt: Pyretroidy są syntetycznymi insektycydami, w swojej strukturze chemicznej zawierają alkohol pierwszo- lub drugorzędowych (zawierających przynajmniej jedno wiązanie podwójne) połączony estrowo z kwasem chryzantemowym [kwasu 3-(2,2-dimetylowinylo)-2,2-dimetylo cyklopropanokarboksylowego] lub analogiem tego kwasu. Związki te znalazły zastosowanie jako insektycydy. Mechanizm ich toksycznego działania na owady polega na hamowaniu aktywności kanałów jonowych w komórkach nerwowych. Według danych literaturowych te pestycydy mogą wywoływać u różnych organizmów szereg niespecyficznych reakcji, których wspólnym mechanizmem może być generowanie reaktywnych form tlenu. Badając toksyczne działanie pyretroidów na komórki drożdży, stwierdzono występowanie mian charakterystycznych dla stresu oksydacyjnego, takich jak: zmiany aktywności katalazy i dysmutazy ponadtlenkowej, zmniejszenie zredukowanego glutationu, zmniejszenie stężenia zredukowanego glutationu i grup tiolowych, obniżenie całkowitej zdolności antyoksydacyjnej. Celem prezentowawnej pracy było zbadanie, czy dodatek antyoksydantów do pożywki uchroni komórki drożdży *Saccharomyces cerevisiae* przed zabiciem powodowanym inkubacją z pyretroidami. Komórki drożdży inkubowano przez 2 h z wybranymi pyretroidami, a następnie wysiewano na pożywkę stałą zawierającą różne antyoksydanty fenolowe, a także witaminy i ich pochodne. Porównywano przeżywalność komórek drożdży na pożywkach kontrolnych i wzbogaconych o antyoksydanty. W przypadku komórek drożdży nie stwierdzono ochronnej roli zastosowanych antyoksydantów przed toksycznością pyretroidów.

Słowa kluczowe: pyretroidy, antyoksydanty, drożdże