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EFFECT OF THE PESTICIDE KARATE 025EC ON THE ANTIOXIDANT PROPERTIES OF RADISH (*Raphanus sativus* L.) SEEDLING EXTRACT

WPŁYW PREPARATU KARATE NA WYBRANE PARAMETRY STRESU OKSYDACYJNEGO W SIEWKACH RZODKIEWKI *Raphanus sativus* L.

Abstract: Radish (*Raphanus sativus* L.) seedlings are a recognized model organism used in short-term ecotoxicological tests, particularly in research on the toxicity of heavy metals, pesticides and xenobiotics. Radish seedlings were used to determine the toxicity of the pyrethroid Karate 025EC. In this study seeds of the Rowa variety of radish (*Raphanus sativus* L.) were germinated in Petri dishes lined with filter paper. The radish seedlings were grown under natural light conditions at a temperature of 22 ^{o}C, with no added nutrients. Samples for analysis were collected 2, 4, and 6 days after plating. Control samples were grown in the presence of water, while in the samples with Karate the pesticide solutions contained 0.005 % to 0.1 % of the active substance. The radish seedlings were shown to be sensitive to lambda-cyhalothrin, the active ingredient in Karate 025EC. The compound inhibited germination of the radish seeds, affected the morphology of the seedlings, and decreased germination energy and rate. The effect depended on the growth time and the concentration of the active substance. Water extracts were prepared from the seedlings that had been subjected to the activity of lambda-cyhalothrin and their antioxidant activity was determined by a modification of the method of Brand-Williams et al, using the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Total antioxidant activity based on reduction of the $ABTS^{\bullet+}$ cation radical was also determined, using the method of Re et al as modified by Bartosz. In the control, the total antioxidant activity of the extracts, determined both by the DPPH method and the ABTS method, decreased with growth time, possibly due to the seedlings rapid growth and utilization of reserve substances for the needs of the developing plant. In the samples grown in the presence of Karate 025EC the antioxidant content in the extract decreased more slowly, and inhibition of seedling growth was observed as well. A description of this phenomenon may be helpful in research on the toxicity of pyrethroids in radish seedlings used as a model organism.

Keywords: pyrethroid, *Raphanus* sp., total antioxidant capacity, ABTS, DPPH

Pyrethroids are crop protection chemicals with insecticidal properties. They block nicotinic acetylocholine receptors and gamma-aminobutyric acid receptors in the nerve cells of insects [1], causing hyperexcitation of the nervous system, irritation of

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respiratory muscles, and inhibition of the functions of the central respiratory centre, leading to the death of the insect [2]. These neurotoxins are characterized by strong insecticidal activity and relatively low toxicity for mammals; they show no tendency to accumulate in living organisms and are quickly metabolized and excreted. Nevertheless, the authors of many studies have emphasized that the mechanism of action of pyrethroids also involves the generation of free radicals, leading to non-specific effects on the cells of various organisms. 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 [3]. For this study on the non-specific effects of pyrethroids, the commercial pesticide Karate 025EC, containing lambda-cyhalothrin, was selected. Various model organisms are used in ecotoxicological tests – microorganisms, nematodes, insects, fish, aquatic invertebrates, and various plant species. The model organism used in this study was radish (*Raphanus sativus* L.) seedlings, which are used in short-term ecotoxicological tests. Studies on the allelopathy mechanism [4] and changes in magnetic field intensity [5] as well as on the toxicity of heavy metals and pesticides [6, 7] have confirmed the usefulness of radish seedlings as a model organism. As germination is a vulnerable moment in plant development, seedlings are much more sensitive to environmental factors than fully developed adult plants.

Materials and methods

The commercial pyrethroid Karate 025 EC, containing lambda-cyhalothrin, was used for the experiments. Water solutions of the pesticide were prepared with 0.005, 0.01, 0.05, and 0.1 % lambda-cyhalothrin content. Seeds of the Rowa variety of radish (*Raphanus sativus* L.) were germinated on Petri dishes lined with filter paper, in natural light conditions, at a temperature of 22 ^{o}C, with no added nutrients [8].

Water extracts from the stems of the radish seedlings were prepared according to the method described by Zielinski and Kozlowska [9]. Determinations were made of total antioxidant capacity in the extracts based on reduction of the $ABTS^{\bullet+}$ cation radical using the method of Re et al as modified by Bartosz [10, 11], and of antioxidant activity based on properties of DPPH using the method of Brand-Williams et al, with modifications [12].

Determination of antioxidant activity using the ABTS method is based on the amount of reduced cation radical $ABTS^{\bullet+}$ produced beforehand in a reaction of ABTS with potassium persulfate. Identical volumes of the radish extracts were added to an $ABTS^{\bullet+}$ cation radical solution. When the antioxidants contained in the extract reduce the $ABTS^{\bullet+}$ cation radical to colourless ABTS, they cause the solution to lose its blue-green colour, with the decrease in intensity of the colour dependent on the antioxidant content in the sample [13]. Absorbance of the solution was measured 30 minutes after the reaction was initiated, at a wavelength of λ = 414 nm [11]. Antioxidant activity was calculated as a percentage of $ABTS^{\bullet+}$ cation radical inhibition using the following equation:

$$\% \text{ inhibition} = 100\ (A_0 - A_{avg.})/A_0 \quad [14]$$

where: $A_{avg.}$ – mean absorbance of the antioxidant solution being tested;
A_0 – absorbance of the radical solution.

The results were expressed as mmol of Trolox per 1 g f.m. of radish hypocotyls.

Using an equation analogous to the one in the method described above, the antioxidant activity of the extracts was calculated using the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Identical volumes of the radish extracts were added to an ethanol solution of DPPH. When DPPH reacts with an antioxidant, the stable DPPH radical takes on electrons from the antioxidant and loses its intense violet colour. The decrease in absorbance was measured in relation to the control sample (DPPH solution + ethanol) 30 minutes after the reaction was initiated at a wavelength of $\lambda = 517$ nm. Antioxidant content was expressed as mmol of Trolox per 1 g f.m.

All determinations were made in at least three independent replications.

Results and discussion

Karate 025 EC inhibits germination and growth of radish seedlings. This effect increased with growth time and depended on the concentration of lambda-cyhalothrin. Because necrotic changes in the radicles were also observed in the plants grown in the presence of higher pyrethroid concentrations, extracts were made from seedling stems with cotyledons. On the fourth day of growth the length of the hypocotyls growing in the presence of the pyrethroid was 16.5, 11.00, 6.70 and 2.20 mm for lambda--cyhalothrin concentrations of 0.005 %, 0.01 %, 0.05 %, and 0.1 %, respectively, compared with the control. The pyrethroid pesticide Cyperkill Super 025EC containing cypermethrin had a similar effect on radish seedlings, reducing germination energy and rate [7]. Radish seedlings are often used in ecotoxicological tests because they are sensitive to various environmental factors. Diamines in concentrations from 0.02 to 0.1 % have been shown to reduce the number of germinating radish seeds [15]. The pesticide 1,2,3,4,5,6-hexachlorocyclohexane (HCH), a chlorinated hydrocarbon, has been found to inhibit growth of radish seedlings [16]. A common manifestation of the phytotoxic effects of various compounds is a decrease in biomass, which was confirmed by Wieczorek in his study on the effect of anthracene on radish seedlings [17].

Apart from their characteristic insecticidal activity, pyrethroids also generate free radicals, leading to symptoms typical of oxidative stress. In studies on the non-specific effects of pyrethroids on various organisms, determinations are often made of such markers of oxidative stress as antioxidant concentration, activity of catalase, superoxide dismutase, and glutathione preoxidase, and lipid peroxidation level. Enzymatic and non-enzymatic cellular protection mechanisms prevent uncontrolled oxidation reactions, particularly free radical reactions. A number of methods are known for determining total antioxidant capacity, understood as the capability of the material tested to counteract a given oxidation reaction. The measurement methods employed in this study, using ABTS and DPPH, enable determination of the total antioxidant capacity of radish hypocotyl extracts.

The methods using the reagents DPPH and ABTS are widely employed for measuring the antioxidant activity of plant extracts, food products [18] and systemic fluids [11]. These methods are often used in determining the antioxidant properties of phenol compounds [19].

When the ABTS method was employed, the total antioxidant capacity of extracts from the two-day radish hypocotyls in the control sample was found to be characterized by high content of antioxidant substances and was equivalent to 98.93 mmol of Trolox (Table 1). In the control samples total antioxidant capacity decreased with the time of the experiment. In 4-day hypocotyls the content of antioxidant substances decreased to about 77 %, and in the 6-day seedlings it was 52 % compared with the 2-day control samples (Table 1). The results obtained when antioxidant properties were measured by the DPPH method also suggest that antioxidant content in the extract decreases during seedling development (Table 1). Antioxidant activity in the extracts from the second day of growth was equivalent to 77.69 mmol of Trolox, while on days 4 and 6 these values were 47.15 and 30.88 mmol of Trolox, respectively.

Table 1

The effect of Karate 025EC on antioxidant properties of extract of radish (*Raphanus sativus* L.) seedlings

Time [day]	Lambda-cyhalothrin concentration [%]				
	0	0.005	0.01	0.05	0.1
Hypocotyl length of radish seedlings [mm]					
4	16.5 (± 6.20)	16.5 (± 4.22)	11.00 (± 5.98)	6.70 (± 3.83)	2.20 (± 2.57)
6	25.50 (± 7.86)	18.40 (± 4.97)	14.40 (± 6.77)	2.10 (± 3.57)	0.2 (± 0.42)
Antioxidant properties of the extract measured by the DPPH method [mmol trolox · g^{-1}]					
2	77.69 (± 9.40)	84.98 (± 5.72)	87.56 (± 7.20)	96.97 (± 3.92)	88.45 (± 6.94)
4	47.15 (± 4.31)	50.68 (± 2.20)	54.40 (± 3.28)	64.52 (± 1.96)	71.78 (± 2.24)
6	30.88 (± 2.56)	41.06 (± 4.94)	41.98 (± 7.94)	50.28 (± 10.96)	38.13 (± 6.08)
Antioxidant properties of the extract measured by the ABTS method of Re et al. [mmol trolox · g^{-1}]					
2	98.93 (± 11.45)	106.93 (± 9.81)	100.95 (± 10.02)	118.02 (± 5.31)	112.32 (± 5.98)
4	75.85 (± 5.39)	75.69 (± 4.90)	77.55 (± 4.33)	85.31 (± 7.56)	101.32 (± 6.52)
6	49.45 (± 5.74)	4.99 (± 9.52)	64.82 (± 13.19)	63,76 (± 17.65)	52.78 (± 10.09)

Measured by the DPPH method, the total antioxidant capacity (TAC) of the extract from the 2-day radish hypocotyls incubated with lambda-cyhalothrin was higher than in the control samples. The changes in total antioxidant capacity characteristic of the control were not found in plants germinating in the presence of the pyrethroid. On day 4 TAC was determined to be 152.23 % for the 0.1 % concentration of lambda--cyhalothrin, and for concentrations of 0.05, 0.01 and 0.005 %, up to about 136.84 %, 115.38 %, and 107.49 %, respectively, where 100 % was the control sample on that day. If we compare the TAC values between days 2 and 6 of growth, the difference (TAC on day 2 – TAC on day 6) is 50.32 for a 0.1 % concentration of lambda-cyhalothrin, and for concentrations of 0.05, 0.01 and 0.005 % it is 46.69, 45.58 and 43.92, respectively.

In the control sample the difference was 46.81. The difference in TAC between days 2 and 4 of growth (TAC on day 2 – TAC on day 4) for concentrations of 0.1, 0.05, 0.01 and 0.005 %, was 16.67, 32.45, 33.16 and 34.3, respectively. In the control sample the difference was 30.54.

In the control, total antioxidant capacity decreased with growth time. TAC of extracts from seedlings grown in the presence of the pyrethroid also decreased, but to a different degree than in the case of the control. When the highest concentration of the pyrethroid, 0.1 %, was used, a significant difference in TAC was observed between days 4 and 6 of growth (in the case of the DPPH method the difference between days 4 and 6 was 33.65 – far more than for other concentrations), while a slight difference was observed between days 2 and 4 of growth (with the DPPH method the difference was 16.67, significantly less than in the case of other concentrations). The changes were similar when the ABTS method was used. This high concentration of lambda-cyhalothrin caused the greatest inhibition of seedling growth, as well as necrotic changes.

The decrease in antioxidant content in the control samples may be due to the high rate of growth of the seedlings. Similar results were obtained when amaranth seedlings were grown; antioxidant content in the extracts decreased between days 4 and 7 of growth [20]. During germination, particularly in the catabolic phase, reserve substances are mobilized and utilized for the needs of the developing seedling [21]. Similar results were obtained in a study on the effect of another pyrethroid preparation, cypermethrin, on seedling development. In radish seedlings growing in the presence of cypermethrin the decrease in antioxidant content was found to take place more slowly than in the control, and cypermethrin also inhibited seedling growth [7]. A description of this phenomenon may be helpful in research on the toxicity of pyrethroids for radish seedlings used as a model organism.

Pyrethroids affect the antioxidant systems of the cells of various, often taxonomically diverse, organisms: microorganisms, invertebrates, mammals and plants. Changes in the functioning of the cellular antioxidant system are a sensitive indicator of oxidative stress induced by pyrethroids. The literature provides many experimental examples confirming this observation. A detailed discussion of this question can be found in several publications by this author [7, 22, 23].

Experiments conducted on *Saccharomyces cerevisiae* yeast have also confirmed that pyrethroids inhibit stress-induced biosynthesis of haemoproteins – catalase and cytochromes [7].

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WPŁYW PREPARATU KARATE NA WYBRANE PARAMETRY STRESU OKSYDACYJNEGO W SIEWKACH RZODKIEWKI *Raphanus sativus* L.

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Abstrakt: Siewki rzodkiewki (*Raphanus sativus* L.) to uznany organizm modelowy, który znalazł zastosowanie w krótkoterminowych testach ekotoksykologicznych, szczególnie w badaniach nad toksycznością metali ciężkich, pestycydów i ksenobiotyków. Siewki rzodkiewki posłużyły do określenia toksyczności preparatu z grupy pyretroidów Karate 025EC. W prezentowanej pracy kiełkowanie nasion rzodkiewki *Raphanus sativus* L. odmiany Rowa przeprowadzono na szalkach Petriego wyłożonych bibułą filtracyjną. Siewki rosły w naturalnych warunkach oświetlenia, w temperaturze 22 ^{o}C, bez dodatku składników odżywczych, próbki do analiz pobierano po 2, 4, 6 dniach od ich wysiewu. Próbki kontrolne rosły w obecności wody, w próbkach z preparatem Karate 025EC zastosowano wodne roztwory tego pestycydu o zawartości substancji aktywnej od 0,005 % do 0,1 %. Stwierdzono, że siewki rzodkiewki są wrażliwe na lambda-cyhalotrynę, aktywny składnik preparatu Karate 025EC. Związek ten hamował kiełkowanie nasion rzodkiewki, wpływał na morfologię siewek, obniżał energię i siłę kiełkowania, a efekt jego działania zależy od czasu hodowli i stężenia substancji aktywnej. Przygotowano wodne ekstrakty z siewek rzodkiewki poddanych działaniu lambda cyhalotryny i oznaczono w nich aktywność antyoksydacyjną według zmodyfikowanej metody Branda-Wiliamsa i współpracowników z użyciem syntetycznego rodnika DPPH (1,1-difenylo-2-pikrylohydrazyl). Całkowitą zdolność antyoksydacyjną opartą na zasadzie redukcji kationorodnika $ABTS^{\bullet+}$ oznaczono według metody Re i współpracowników w modyfikacji Bartosza. W kontroli w miarę czasu hodowli całkowita zdolność antyoksydacyjna ekstraktów oznaczana zarówno metodą z DPPH, jak i z ABTS zmniejsza się, co może być powiązane z intensywnym wzrostem siewek i wykorzystaniem substancji zapasowych dla potrzeb rozwijającej się rośliny. W próbkach rosnących w obecności preparatu Karate 025EC obniżanie się ilości antyoksydantów w ekstrakcie zachodzi wolniej, w próbkach tych stwierdzono też zahamowanie wzrostu siewek. Opis tego zjawiska może być pomocny w badaniach nad toksycznością pyretroidów na organizmie modelowym, jakim są siewki rzodkiewki.

Słowa kluczowe: pyretroidy, *Raphanus* sp., całkowita zdolność antyoksydacyjna, ABTS, DPPH