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EFFECT OF BETOKSON SUPER AND FUSILADE PREPARATIONS AND INDOLEACETIC ACID (IAA) ON SOME PHYSIOLOGICAL PROCESSES OF THE RADISH (*Raphanus sativus* L.) AND THE YEAST (*Saccharomyces cerevisiae*)

ODDZIAŁYWANIE PREPARATÓW HERBICYDOWYCH: BETOKSON SUPER, FUSILADE ORAZ KWASU INDOLILOOCTOWEGO (IAA) NA WYBRANE PROCESY FIZJOLOGICZNE RZODKIEWKI (*Raphanus sativus* L.) ORAZ DROŻDŻY (*Saccharomyces cerevisiae*)

Abstract: Studies of non-specific effect of herbicides concern not only plants but also other organisms, mostly those which are directly exposed to the influence of herbicides in the natural environment. Reports on studies concerning a reaction on herbicides of phylogenetically distant organisms are more and more frequent. The author of this study examined the effect of commercial pesticide preparations Betokson super containing β-naphthaleneacetic acid (NOA), Fusilade containing fluazifop-*p*-butyl and indoleacetic acid (IAA) on selected parameters of the process of germination of the radish (*Raphanus sativus* L.) seeds and the growth of the yeast (*Saccharomyces cerevisiae*) cells. In the case of radish the effect of these preparations on the dynamics of seed germination and elongation of seedlings was analysed. In the case of yeast, the effect of the preparations on the dynamics of growth of cells in the wild strain in liquid medium was examined.

The experiments show that sprouts and seedlings are the most sensitive to the examined preparations; significant inhibition of elongation of seedling organs was observed in the presence of examined preparations in concentrations lower than 10 $\mu g \cdot cm^{-3}$. In this case it was observed that the strongest effects were induced by Betokson super preparation. The dynamics of sprouting of radish seeds and growth of yeast cells did not undergo significant changes under these conditions. Distinct inhibition of the process of sprouting and cell divisions in yeast was observed after adding the examined preparations in concentrations higher than 10 $\mu g \cdot cm^{-3}$. The parameters which characterise the growth of yeast cells may be used in studies of non-specific effect of herbicides.

Keywords: yeast, radish, NOA, IAA, fluazifop-*p*-butyl

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The studies concerning the toxicity of herbicides concentrate not only on plant organisms but also include other living organisms and particularly those organisms which are directly exposed to the herbicides in the natural environment. Reports on studies concerning the response to herbicides of phylogenetically distant organisms are more and more frequent, eg the studies concerning the toxicity of herbicides conducted by the Papaefthimiou et al [1] concerned two different test systems, one based on the isolated sciatic nerve of amphibian and the other on a microbial eukaryote. The studies conducted by the Venkov team [2] concerned the reaction of three tests based on yeast, transformed hematopoietic and mouse bone marrow cells. Comparing the response to auxin herbicides, the studies conducted by Fargasova [3] consisted of four biological subjects (*Daphnia magna*, *Tubifex tubifex*, *Scenedesmus quadricauda* and seeds of *Sinapis alba*).

In the study the yeast cells of *Saccharomyces cerevisiae* were chosen as well as the seeds and young seedlings of radish (*Raphanus sativus* L.) Both radish and yeast are model organisms commonly used for studies concerning the effect of xenobiotics, such as heavy metals or pesticides [4–8]. Tests based on measuring the sensitivity of the germination process and elongation of radicles are used in short-term ecotoxicological studies [9].

The purpose of the present study was to determine the effect of pesticide preparations (Betokson super, Fusilade) containing β-naphthaleneacetic acid (NOA) and fluazifop--*p*-butyl, respectively, as active substances and indoleacetic acid (IAA) on the selected parameters of the germination process on the seeds of radish (*Raphanus sativus* L.) and the growth of cells of yeast (*Saccharomyces cerevisiae*). In order to compare the results obtained for these two different organisms, the level of inhibition of the parameters in study was determined.

Material and methods

Seeds of radish (*Raphanus sativus* L.), 'Rowa' cv., from Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa Ożarów Mazowiecki were used in the experiment.

Radish seeds were sowed in 50-seed batches on Petri dish padded with circles of filter paper. The medium for germination was a water solution of herbicide preparations of Betokson super, Fusilade and indoleacetic acid (IAA) in the following concentrations: 2, 4, 6, 8, 10, 20, 30 $\mu g \cdot cm^{-3}$. Stock IAA in concentration of 100 $\mu g \cdot cm^{-3}$ was made by dissolving the appropriate amounts of IAA in DMSO. The dishes with seeds were incubated in an incubator at 25 ^{o}C in the dark.

After 24, 36 and 48 hours the number of germinated seeds was determined. The appearance of a radicle protruding to more than 2 mm was accepted as a criterion for germination [10]. After 48 hours from sowing the length of roots, hypocotyls and whole seedlings was measured with a ruler. Root, hypocotyl and whole seedling elongation inhibitory rates were calculated as differences between vegetables exposed to herbicides or IAA and the control sample cultured in distilled water. The inhibitory rates of the process of seed germination were determined in the same way.

The sensitivity of yeast cells of the SP4 wild strain of genotype Mat α leu1 arg4 to the examined herbicide preparations was determined by measuring the density of the culture [11]. The yeast cultures were grown on YPD liquid medium containing: 10 g · dm^{-3} of yeast extract, 10 g · dm^{-3} of peptones, 20 g · dm^{-3} of glucose and different concentrations of Betokson super and Fusilade preparations and indoleacetic acid (IAA). The cultures were incubated on a rotary shaker at 28 ^{o}C. The density of the cultures was determined after 24 and 48 hours from inoculation by counting the cells in the Malassezz hemocytometer chamber. The level of inhibition of the growth of yeast cells was calculated as a difference between the density of the culture conducted in the presence of herbicide preparations and the control sample cultured in medium which did not contain any supplements.

The herbicide preparations used in the experiments were bought in shops selling crop protection products. Indoleacetic acid (IAA) was bought in Sigma. The presented results are average values from at least 3 experiments conducted independently. After data were obtained from the experiments, average values and standard deviations (SD) were calculated.

Results

At the first stage of the experiment, there was determined the dynamics of germination of radish seeds in the presence of selected concentrations of herbicide preparations of Betokson super, Fusilade containing β-naphthaleneacetic acid (NOA) and fluazifop-*p*-butyl as active substances, respectively and indoleacetic acid (IAA) (Table 1). On the basis of obtained data the rates of inhibition of germination process were calculated as a difference between the percentage of germinated seeds in the control and the seeds in the samples with herbicides or IAA.

Table 1

Effect of herbicides preparations and YAA on the dynamics of germination of radish (*Raphanus sativus* L.) seeds

Concentrations of active substances [μg · cm^{-3}]	Germinated seeds [%]					
	After 24 hours from sowing	Inhibition rate [%]	After 36 hours from sowing	Inhibition rate [%]	After 48 hours from sowing	Inhibition rate [%]
0 (control)	50.3	0	62.8	0	80.1	0
NOA						
2	54.0	0	63.4	0	87.0	0
4	63.4	0	68.2	0	76.3	4.7
6	62.0	0	68.5	0	78.4	2.6
8	62.0	0	62.0	1.3	82.1	0
10	56.3	0	68.1	0	80.2	0
20	62.1	0	63.0	0	78.5	2.6
30	51.8	0	64.2	0	73.7	8.8
Fluazifop-*p*-butyl						
2	60.5	0	67.3	0	76.1	0
4	66.6	0	69.3	0	80.4	0

Table 1 contd.

Concentrations of active substances [μg · cm^{-3}]	Germinated seeds [%]					
	After 24 hours from sowing	Inhibition rate [%]	After 36 hours from sowing	Inhibition rate [%]	After 48 hours from sowing	Inhibition rate [%]
6	67.3	0	68.0	0	85.2	0
8	72.0	0	74.1	0	86.0	0
10	55.2	0	64.2	0	85.2	0
20	42.5	15.5	63.1	0	80.8	0
30	30.1	40.1	45.1	28.2	56.7	29.2
IAA						
2	53.1	0	65.4	0	82.6	0
4	45.5	9.5	55.5	11.6	79.0	1.4
6	46.8	6.9	56.4	10.2	84.2	0
8	45.4	9.7	54.4	13.4	76.2	4.9
10	44.0	12.5	52.3	16.7	77.2	3.6
20	38.6	23.2	49.3	21.5	76.4	4.6
30	36.3	27.8	45.6	27.4	68.4	14.6

Inhibition of the germination process of radish seeds was the largest in the presence of exogenous indoleacetic acid (IAA). Fusilade preparation affected this parameter to a smaller degree. Only the largest concentrations of this substance (20 and 30 μg · cm^{-3}) led to an evident inhibition of the seed germination process. As far as Betokson super is concerned, it had almost no effect on the dynamics of germination of radish seeds. It should be stressed that negative effects of the examined preparations were more evident at the initial stage of seed germination (24 hours from sowing) than at later stages.

Next, the effect of the selected herbicide preparations and IAA on the length of radish seedlings and its organs (root, hypocotyl) was examined. Measurements were taken after four days from sowing the seeds. The obtained results are presented in Table 2. The inhibition rates of elongation of these organs and of whole seedlings were calculated as a difference between a mean length of radicle, hypocotyl and seedling from control seeds and a mean length of radicle, hypocotyl and seedling of seeds grown in the presence of herbicides or IAA.

The mean length of seedlings which developed in the presence of Fusilade, Betokson super and concentrations of IAA higher than 2 μg · cm^{-3} was smaller than the average length of seedlings in the control. The highest level of inhibition of seedling elongation was observed for Betokson super containing NOA as an active substance. In the case of exogenous indoleacetic acid used in small concentrations the mean length of seedlings was larger than in the control, which suggests that this compound stimulates seedling elongation.

While comparing the effect of the examined preparations on the process of elongation of the particular organs of seedlings it was observed that the examined preparations and exogenous indoleacetic acid inhibited the elongation of roots more than the elongation of hypocotyl.

The inhibition of root elongation was very big in the case of all examined concentrations of Betokson super. Similar effect was obtained only after the application

of the highest concentration of Fusilade. When the concentration of Fusilade was low, the length of hypocotyl was medium, in spite of the fact that the mean length of roots and the whole seedling was reduced in these conditions. After application of low concentration of IAA (2 μg · cm^{-3}) the mean length of the seedling and the length of its organs was bigger.

Table 2

Effect of herbicide preparations and IAA on the length of radicles, hypocotyls and seedlings of radish (*Raphanus sativus* L.)

Concentrations of active substances [μg · cm^{-3}]	Mean length of seedlings and their organs ± standard deviation [mm]					
	Radicle	Inhibition rate [%]	Hypocotyl	Inhibition rate [%]	Seedling	Inhibition rate [%]
0 (control)	47.98 ± 2.5	0	13.9 ± 3.6	0	61.9 ± 5.3	0
NOA						
2	10.0 ± 3.1	79.1	6.8 ± 2.1	51.1	16.8 ± 2.4	72.8
4	6.9 ± 2.3	85.5	5.5 ± 2.8	59.9	12.5 ± 3.4	79.7
6	6.3 ± 1.4	86.8	5.4 ± 2.7	60.6	11.7 ± 2.1	80.9
8	4.2 ± 3.1	91.1	6.0 ± 1.8	56.4	10.2 ± 1.4	83.5
10	3.7 ± 2.4	92.1	5.1 ± 2.2	62.8	8.8 ± 2.4	85.5
20	3.1 ± 1.3	93.3	5.0 ± 1.6	63.8	8.1 ± 1.1	86.7
30	1.6 ± 1.3	96.5	4.1 ± 0.9	70.2	5.7 ± 0.6	90.5
Fluazifop-*p*-butyl						
2	37.4 ± 4.2	22.0	14.7 ± 4.2	0	52.1 ± 8.1	15.8
4	36.6 ± 5.2	23.6	15.5 ± 3.5	0	52.1 ± 4.1	15.8
6	38.8 ± 4.1	19.0	16.0 ± 6.4	0	54.8 ± 4.8	11.4
8	38.4 ± 3.8	20.8	18.1 ± 3.2	0	56.1 ± 2.4	9.2
10	41.3 ± 4.9	13.9	15.1 ± 4.7	0	56.4 ± 4.5	8.8
20	39.8 ± 2.1	16.9	13.5 ± 2.5	2.3	53.4 ± 3.9	13.6
30	4.5 ± 1.0	90.6	3.0 ± 0.8	78.1	7.5 ± 1.3	87.7
IAA						
2	54.3 ± 6.3	0	16.8 ± 5.3	0	71.2 ± 4.4	0
4	45.6 ± 2.1	4.8	13.6 ± 2.5	2.1	59.2 ± 5.6	4.3
6	40.0 ± 5.9	16.6	16.3 ± 3.7	0	56.4 ± 7.1	8.9
8	35.8 ± 6.3	25.2	12.9 ± 3.3	6.8	48.8 ± 4.2	21.1
10	36.9 ± 3.1	23.1	11.2 ± 1.5	18.9	48.1 ± 3.5	22.2
20	23.0 ± 2.7	51.9	9.3 ± 1.3	32.0	32.4 ± 4.2	47.6
30	21.4 ± 5.6	55.3	10.6 ± 2.4	23.7	32.0 ± 1.2	48.3

Next, there was examined the effect of herbicide preparations of Betokson super, Fusilade and IAA on the growth of yeast cells of the SP4 wild strain. Yeast cells were grown in standard conditions on liquid medium (YPD) supplemented with the concentrations of herbicide preparations and IAA presented in Table 3.

Young yeast cultures (24 hours) were the most sensitive to Fusilade preparation. After the cells were treated with concentrations of this preparations exceeding 10 μg · cm^{-3}, the density of the cultures was much smaller than in the control. The highest level of inhibition of cell growth was observed after the application of the biggest concentrations of the herbicide (100 and 200 μg · cm^{-3}). The other two preparations

inhibited the growth of yeast cells when added in concentrations of 50 μg · cm^{-3} and higher. Older cultures (48 hours) were sensitive to Betokson super and Fusilade when these preparations were applied in concentrations exceeding 50 μg · cm^{-3}. Even applied in the highest concentration of 200 μg · cm^{-3} exogenous indoleacetic acid reduced the growth of yeast cells only to a small extent.

Table 3

Effect of Betokson super, Fusilade and IAA preparations on the dynamics of growth of yeast cells

Concentrations of active substances [μg · cm^{-3}]	Density of yeast cells culture of wild strain SP4			
	After 24 h from inoculation [cells · 10^6 · cm^{-3}]	Inhibition rate [%]	After 48 h from inoculation [cells · 10^8 · cm^{-3}]	Inhibition rate [%]
0 (control)	22.3	0	3.2	0
NOA				
10	26.7	0	5.4	0
20	23.4	0	5.5	0
50	3.9	82.5	3.0	6.25
100	0.4	98.0	0	100
200	0	100	0	100
Fluazifop-*p*-butyl				
10	19.1	14.3	3.8	0
20	17.6	21.1	4.6	0
50	10.1	54.7	3.0	6.2
100	4.0	82.1	1.6	50.0
200	2.7	87.8	0.9	71.9
IAA				
10	22.0	1.3	6.1	0
20	23.8	0	4.6	0
30	22.9	0	4.3	0
50	21.2	4.9	5.7	0
100	16.4	26.4	4.5	0
200	7.7	65.4	2.3	28.1

Discussion

Auxin is a plant hormone which regulates and influences many aspects of the growth and development of plants. Treatment of plants with exogenous IAA can result in a variety of physiological and morphological effects. One of the symptoms of treatment of Arabidopsis seedlings with natural or synthetic auxins is inhibition of root growth [12].

The obtained results show that exogenous IAA (4-(indol-3-yl)acetic acid) in concentration higher than 2 μg · cm^{-3} inhibits the elongation of radicle and hypocotyl. Only when IAA was applied in concentration of 2 μg · cm^{-3}, small effect stimulating the germination of radish seeds and elongation of seedlings was observed.

Yeast cells were more resistant to the effect of this phytohormone. Negative effects consisting inhibition of yeast cell division were visible after an application of about ten times higher doses than the doses effective for radish seedlings. These results agree with

the data obtained by Prusty et al [13]. According to them, the application of higher concentrations of IAA inhibits the growth of yeast strains both in liquid and solid media. In this case the concentration inducing 50 % inhibition of growth measured as the OD growth in the culture was EC_{50} = 250 μM, which corresponds to about 43 $\mu g \cdot cm^{-3}$. In the present study the effective concentrations were higher both for logarithmic and for stationary cells. The differences are caused most probably by a different sensitivity of the wild strains used for the experiments, which differ from each other in the genetical backgrounds.

According to Prusty et al [13] IAA arrests yeast growth at all stages of the cell cycle and after removal of IAA the cells resume growth. At lower concentrations it induces filamentation and adhesion (invasive growth of yeast cells). These responses are mediated by family of transporters and the fungal transcription factor Yap1p, which is engaged in the response of yeast cells to oxidative stress. IAA also inhibits the growth of the *Ustilago maydis*, a fungal pathogen of corn.

NOA (β-naphthaleneacetic acid) is a synthetic derivative of auxin. In high concentrations (herbicide doses) synthetic auxins induce various defects of plant growth, such as stem curvature, leaf epinasty, inhibitions of root and shoot growth, decrease in leaf surface and opening of stomata. These defects lead to a decrease in transpiration, carbon assimilation, ageing and local necroses causing death in the end [14]. The results of our studies show that the process which is the most sensitive to NOA effect is elongation of radish seedlings (particularly the elongation of the radicle). The process of seed germination was inhibited only when the concentration of this substance was 10 times higher. The same concentration of NOA was also effective in the case of young, logarithmic yeast cells.

The results presented in this study show that natural auxin has a smaller inhibiting effect on root growth than its synthetic analogue NOA (Table 2).

According to the studies conducted by Walsh et al [15] a variety of synthetic auxins and natural auxins IAA are characterized by a different potency in the reduction in *Arabidopsis* root growth. Out of the compounds examined by the authors (novel picolinate auxin DAS534, 2,4 D and IAA) natural auxin also had the smallest inhibiting effect on root growth.

Fluazifop-*p*-butyl is an active ingredient of APS (aryloxyphenoxypropionates herbicides). They inhibit the lipid synthesis in plant by interfering with the activity of the enzyme acetyl-coenzyme A carboxylase (ACCase), acting on the meristematic level.

The results of the experiments presented in this study show that the parameter which was the most sensitive to the effect of this herbicide was the process of elongation of seedlings and radicles. The process of germination of seeds, just like the division of yeast cells were inhibited only when the doses of this herbicide were 5 times higher.

While comparing the response of organs of seedlings it should be stressed that the elongation of radicles was the most sensitive process to the effect of all the examined substances. It agrees with the observations of other researchers. While examining the effect of pollutants, such as cadmium, copper and chlorimuron-ethyl (used as herbicides) Wang and Zhou [16] found that the sensitivity of wheat to the toxicity of the three pollutants was in the following sequence: root elongation > shoot elongation >

> germination rate. Cheng and Zhou [17] and Song et al [18] identified in their studies concerning the toxicity of heavy metals (Cd, Cu, Pb, Zn) and a chemical (reactive X-3B red dye) to wheat that the inhibitory rate of root elongation was higher than the germination rate with the same concentration of pollutants. A similar relation was also identified in radish while comparing the sensitivity of organs of seedlings to sodium benzoate or Al-based coagulants.

Conclusions

The results showed that root elongation was a more sensitive indicator than seed germination and proliferation rate of yeast for evaluating the toxicity of herbicide preparations. But the parameters which characterise the growth of yeast cells may be used in studies of nonspecific effect of herbicides.

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Abstrakt: Badania dotyczące niespecyficznych oddziaływań pestycydów koncentrują się nie tylko na organizmach roślinnych, ale także obejmują inne organizmy, szczególnie te które są bezpośrednio narażone

na ich działanie w środowisku naturalnym. Coraz częściej spotyka się relacje z badań dotyczących odpowiedzi na herbicydy odległych filogenetycznie organizmów. W prezentowanej pracy badano oddziaływanie preparatów pestycydowych Betokson super (zawierający kwas β-naftoksyoctowy, NOA), Fusilade (zawierający fluazifop-*p*-butylowy) i heteroauksyny (IAA) na wybrane parametry procesu kiełkowania nasion rzodkiewki (*Raphanus sativus* L.) oraz wzrostu komórek drożdży (*Saccharomyces cerevisiae*). Oznaczono wpływ badanych substancji na dynamikę kiełkowania nasion i wydłużanie organów siewek rzodkiewki oraz na dynamikę wzrostu komórek drożdży w pożywce płynnej. Najbardziej wrażliwe na badane substancje okazały się kiełki i młode siewki. Wysoki poziom inhibicji wydłużania organów siewek zanotowano dla mniejszych niż 10 $\mu g \cdot cm^{-3}$ stężeń substancji aktywnych badanych preparatów. Najsilniejsze efekty hamowania wydłużania siewek i korzeni zaobserwowano po zastosowaniu preparatu Betokson super. Natomiast dynamika kiełkowania nasion rzodkiewki i przyrost liczby komórek drożdży w tych warunkach nie ulegały znaczącym wahaniom. Wyraźne hamowanie procesu kiełkowania i podziałów komórkowych drożdży zaobserwowano dopiero po zastosowaniu większych niż 10 $\mu g \cdot cm^{-3}$ stężeń badanych substancji. Parametry charakteryzujące wzrost komórek drożdży mogą być wykorzystywane w badaniach nad niespecyficznymi oddziaływaniami herbicydów.

Słowa kluczowe: drożdże, rzodkiewka, NOA, IAA, fluazifop-*p*-butylowy