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Prooxidant–antioxidant system and change of alkaloids level in jimson weed (*Datura stramonium* L.) under the influence of static magnetic field

Wpływ stałego pola magnetycznego na układ prooksydacyjny i antyoksydacyjny oraz zmianę zawartości alkaloidów w bieluniu dzięd zierzawie (*Datura stramonium* L.)

Summary. The effect of magnetic radiation on the prooxidant-antioxidant system and alkaloids level in Jimson weed (*Datura stramonium* L.) have never been studied and reported before. The objective of this research was to study the influence of static magnetic field on the homeostasis and alkaloids level in *D. stramonium*. The level of (thio) barbituric acids active products, superoxide dismutase and catalase activity in *D. stramonium* seedlings under the influence of static magnetic field with the intensity of 3 mT and 10 mT at two exposure times, 30 min and 22 hours, was studied. Under these conditions, the level of alkaloids in the extract from leaves and stems of germs was determined by chromatographic analysis. An increased level of lipid peroxidation products, the activity of superoxide dismutase and catalase in seedlings under the long-term effect of magnetic field were observed. The level of scopolamine alkaloid depended on the duration of exposure to the static magnetic field; however, this factor did not effect the atropine level. The obtained experimental data was confirmed by the results of the analysis of variance.

Key words: *Datura stramonium*, static magnetic field, lipid peroxidation, superoxide dismutase, catalase

INTRODUCTION

The local and global changes in the magnetic field as a result of human activity in the environment are observed. The influence of magnetic field on living objects, including plants, is an important direction in the current researches [Scrape et al. 2008, Jinapang et al. 2010, Payez et al. 2013]. Substantial magnetic field parameters which determine its effect on living objects are intensity and duration of exposure of the influence. The high magnetic flux density (to 150 mT) and a significant duration of exposure (more than18 hours) inhibits plant growth and development, in general [Jinapang et al. 2010, Payez et al. 2013]. The low magnetic fields (0.05–0.5 mT) and short-term action stimulates the seed germination and growth processes, including such plants as Cicer arietinum, *Datura stramonium*, *Anthemis monantha* and Foeniculum [Scrape et al. 2008, Vashisth et al. 2008]. The magnetic field with intensity of 4mT and duration of exposure of 30 min stimulates the seed germination of wheat and its further development. However, the long–term effect of the field with the same intensity leads to abnormal changes in the root system and shoots of the plants [Cakmak et al. 2010, Vashisth et al. 2010, Cakmak et al. 2012]. The longer the period from sowing to germination of seeds, the greater the likelihood of microbial or fungal damage of plants and its death [Scrape et al. 2008].

To clarify the mechanism of the influence of a static magnetic field (SMF) on the plants, the lipid peroxidation (LPO) and enzyme activity of antioxidant system in the cells was studied by Shine et al. [2012]. The magnetic field with intensity of 150 and 200 mT and duration of exposure of one hour stimulates the formation of reactive oxygen in cells of soybean [Shine et al. 2012]. Increased level of active forms of oxygen leads to the increase of antioxidant system activity which is the early response of the plants under the stress factor effect [Baran 1998, Sahebjamei et al. 2007, Shine et al. 2011, Anand et al. 2012]. This mechanism is the basic of the plants adaptations to the external factors [Mamenko et al. 2014].

Preseeding processing of cucumbers seeds by static magnetic field leads to the increasing of superoxide radicals by 41% and hydrogen peroxide by 8% compared with the control group of plants [Bhardwaj et al. 2012]. The influence of the magnetic field with intensity of 10 mT and 30 mT causes the increase of superoxide dismutase activity, the intensification of lipid peroxidation and decreased activity of catalase and peroxidase in tobacco seedlings [Sahebjamei et al. 2007].

The magnetic field can cause genes disruption, structural changes of the protein molecules and enzymes that have a negative effect on antioxidant system of the cells [Baran et al.1998, Shine et al. 2011, Anand et al. 2012].

However, the SMF effect on the growth, metabolism and activity of prooxidant–antioxidant system in the cells depends not only on the parameters of the magnetic field but it also depends on the genus or species of the studied plant [Scrape et al. 2008]. In view of the above described facts, the aim of this research was to perform the comprehensive study of the static magnetic field influence on the growth, prooxidant–antioxidant homeostasis of the cells and the alkaloids level in the extract from *Datura stramonium* seedlings.

MATERIAL AND METHODS

Datura stramonium is a valuable medicinal plant with anti-inflammatory, antispasmodic properties and is used as the raw material for obtaining of atropine and scopolamine alkaloids. *Datura stramonium* seeds collected in the Botanical garden of Ivan Franko Lviv National University has been used for the experimental part of this research.

The dried seeds of *Datura stramonium* were subjected to the effect of the static magnetic field with intensity of 3 mT and 10 mT and duration of exposure 30 min and 22 hours. After exposure seeds were placed on Petri dish covered by filter paper, wetted by distilled water, and cultivated in thermostat with the constant intensity of light (1000 lux) at temperature +30°C (the optimum temperature for germination of seeds of *Datura stramonium*) until germination. The sprouted seeds were transferred to aquatic environment (contains Ca $(NO_3)_2$, KH_2PO_4, KCl, $MgSO_4$ and $FeCI_3$) [Scrape et al. 2008] and kept for 30 days at a temperature of 22–24°C (the optimum temperature for growth of seedlings *Datura stramonium*) under natural light. Intact seeds grown in similar conditions were used as a control.

The germinated seedlings were weighed (1 ±0.9 g) and homogenized in a porcelain mortar with buffer solution in a ratio of 1:10 as is described in the paper [Bilchuk et al. 2014]. The obtained homogenates were centrifuged for 20 min at 15.000 *g* (Thermo Pico 17, Germany). Obtained supernatant was used as a source of the enzymes for studying the enzymatic activity. In total 1 ml of the supernatant from each sample was used for research. Protein level of each sample was determined by the Lowry method [Lowry et al. 1951].

The level of (thio) barbituric acid (TBA) -active products in the supernatant from *D. stramonium* seedlings was determined as described in the paper [Timirbulatov et al. 1981] and expressed as μmol of malondialdehyde (MDA) × $mg^{-1}$of protein. The determination of superoxide dismutase and catalase activity was carried out by methods described in the paper [Kostiuk et al. 1990, Korolyuk et al. 1988].

For the chromatographic analysis, the homogenized sample of 0.1 g was placed to the glass sample bottle and filled with methanol in a ratio of 1:10. The mixture was mixed and extracted at room temperature (+25°C) during 2 hours. The obtained solutions were thoroughly mixed and filtered. In total, 1 ml of filtrate was taken for separation and identification of the components on the system chromatography-mass spectrometry (Agilent Technologies, United States) contained of a gas chromatograph 6890N and mass selective detector 5975V. The chromatographic separation was performer using capillary column NR-5ms with the length of 30 meters and inner diameter 0.25 mm, phase 0.25 microns and column thermostat initial temperature +50°C. Exposure at the initial temperature was 2 minutes. Programmed temperature was increasing from +50°C to +200°C at a speed of 10°C/min, from 200°C to 300°C at a speed of 20°C/min. The final temperature isotherm was 8 minutes helium carrier gas with a flow rate of 1.0 mL/min, automatic supply sample injector 7683V, mass selective detector, interface temperature was 280°C. Ionization was achieved by electron impact, where an ionization energy was 70 eV and temperature of ion source 230°C. Quadrupoles temperature was 150°C. Data collecting speed (frequency) was 0.5 seconds in the mass range of 30–500 m. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. Identification of the products was carried by using

AMDIS program (www.chemdata.nist.gov/mass-spc/amdis), and compared with the mass spectra catalogs Wiley and NIST2008.

Statistical analysis of the results was carried out using Microsoft Excel (2010) program for determination of the probability of difference between the arithmetic mean of the two sets of the data. The difference between the studied parameters was considered as a statistically significant at $P < 0.05$. Two-factor ANOVA with repetition as well as the percentage share of factor influence calculation were carried out to assess (evaluate) the influence of the changes in the duration of exposure and intensity of SMF on the level of lipid peroxidation products as well as superoxide dismutase and catalase level in the cell, and the percentage of atropine and scopolamine in the extract.

RESULTS AND DISCUSSION

To understand the influence of SMF on *D. stramonium* seeds, the level of TBA-active products as well as the activity of antioxidant system enzymes in the seedlings, including superoxide dismutase and catalase, were analyzed. The dependence of the level of TBA-active products on the change of the duration of exposure and intensity of the SMF effect in *D. stramonium* seeds was investigated (Table 1). The maximum level of TBA-active products was observed under the influence of SMF intensity of 3 mT and duration of exposure of 22 hours. A significant increasing of the level of TBA-active products was also observed under the SMF intensity of 10 mT and duration of exposure of 22 hours. The level of TBA-active products was not changed compare to control, not significantly different from control under the effect of 3 mT and 10 mT SMF intensity and duration of exposure of 30 min.

Thus, the maximum SMF effect on the level of TBA-active products in *D. stramonium* seedlings was observed at the influence of 3 mT and 10 mT SMF intensity with the duration of exposure of 22 hours. These parameters were not changed for short-term SMF intensity. These results indicate the violation of the redox balance during the long-term influence of SMF on *D. stramonium* seeds.

Table 1. The level of TBA-active products in *Datura stramonium* seedlings under the influence of SMF

Tabela 1. Poziom aktywnych produktów TBA w sadzonce *Datura stramonium* pod wpływem stałego pola magnetycznego

The studied factors Badane czynniki		The level of TBA-active products Poziom aktywnych produktów TBA (μmole of MDA × mg^{-1} of protein)
Control/ Kontrola		0.326 ±0.003
30 min	3 mT	0.349 ±0.01
	10 mT	0.322 ±0.01
22 h	3 mT	0.480 ±0.013***
	10 mT	0.402 ±0.013*

The statistical significance is * $P < 0.05$, *** $P < 0.001$, compared to the control

Istotność różnic przy * $P < 0,05$, *** $P < 0,001$ w porównaniu z kontrolą

Table 2. Superoxide dismutase and catalase activity in *D. stramonium* seedlings under the SMF effect
Tabela 2. Wpływ stałego pola magnetycznego na aktywność dysmutazy ponadtlenkowej i aktywność katalazy w ekstraktach z *D. stramonium*

The studied factors Badane czynniki		Enzyme / Enzym	
		superoxide dismutase dysmutaza nadtlenkowa (activity unit / mg of protein)	catalase katalaza (µmol of H_2O_2 x mg of protein)
Control/ Kontrola		59.68 ±2.28	0.07 ±0.02
30 min	3 mT	51.10 ±2.68 *	0.03 ±0.01
	10 mT	58.38 ±1.65	0.13 ±0.01*
22 h	3 mT	79.00 ±2.51***	0.34 ±0.03***
	10 mT	65.54 ±1.44*	0.16 ±0.02**

The statistical significance is * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the control
Istotność różnic przy * $P < 0,05$, ** $P < 0,01$, *** $P < 0,001$ w porównaniu z kontrolą

The metabolic processes during seed germination effects the further development of the seedlings. Therefore, the impact of any stressors during seed germination can be displayed on the further development of the plant [Sahebjamei et al. 2007]. Active forms of oxygen are always present in plant organism and can interact with different biological compounds to form free radicals. Formation of the active forms of oxygen (AFO) under the influence of physical factors of different origin *in vivo* can enhance adaptation to the extreme conditions. However, imbalance between the creation and disposal of AFO in the cells leads to oxidative stress. Formation of catalase and superoxide dismutase is the main mechanisms for protection of the cells from the oxidative stress. The studies of their activity can be useful for explanation the possible mechanism of SMF effect on living organism.

The increased activity of superoxide dismutase and catalase in *D. stramonium* seedlings under the long-term influence of SMF was observed. This result is consistent with the results of TBA-active products level (Table 2). The maximum activity of the enzymes was detected under the influence of 3 mT and 10 mT SMF intensity and duration of exposure of 22 hours compared to the same intensity with duration of exposure of 30 min. Under these conditions, the activity parameters of superoxide dismutase and catalase were 1.31 times higher and 4.8 times higher ($P < 0.001$), respectively. It should be noted that inhibitory effect of SMF on the enzymes was observed at the intensity of 3 mT.

Thus, a long-term 3 mT and 10 mT SMF intensity causes the increased activity of catalase and superoxide dismutase. The optimal intensity is 3 mT SMF. These data are consistent with those which described by Sahebjamei et al. [2007]. Similar to our results, the increased activity of superoxide dismutase and catalase activity was detected after long-term influence of magnetic field on suspension-cultured of tobacco cells. The increased level of TBA-active products indicates the initiation of lipid peroxidation process, activation of superoxide dismutase and catalase by long-term influence that confirms the adaptation process [Novitski et al. 2011, Mamenko et al. 2014].

Despite the long history of the studies of magnetic field effect on living organisms the main mechanism of it remains unclear [Bingi et al. 2003, Novitski et al. 2011]. According to one of the hypotheses, there are several magneto sensitive stages in the reactions of lipid peroxidation that can affect lipids metabolism. SMF can also affect the recombination rate of lipid radicals [Novitski et al. 2011].

The Larmor's theorem studied by Fesenko and Edmonds was used to explain one of the potential influences of magnetic field on living organisms [Bingi et al. 2003]. The activity of antioxidant system enzymes depends on Fe^{3+}, Zn^{2+} and Cu^{2+}ions in their active center. The central idea of Larmor's theorem says that direction of ion oscillation in the enzyme active center has a decisive influence on its form and can cause to changes in enzyme activity [Bingi et al. 2003]. The experimental results show disturbance in the cell homeostasis and oxidative stress. However, the increased activity of superoxide dismutase and catalase indicates adaptive response of the cells under the effect of permanent residence [Novitski et al. 2011]. Perhaps, this adaptive response to the long-term exposure of SMF is predetermined by its effect on the lipid membranes of cells. Perhaps, the adaptive effect can change the rate of lipid radical recombination, which leads to the accumulation of lipid peroxidation products [Novitski et al. 2011].

Datura stramonium is valued in medicine as a source of alkaloids, such as atropine (blocker of M-cholinergic receptors) and scopolamine (antagonist of muscarinic receptors) [Iranbakhsh et al. 2006]. The relative percentage of these alkaloids under the effect of different SMF intensity and duration of exposure in *D. stramonium* extract was studied (Table 3). A significant increase of scopolamine was observed under the 3 mT SMF intensity during 22 hrours exposure. However, relative percentage of this alkaloid was significantly decreased under the effect of 3 mT and 10 mT during 30 min. Interestingly, in the case of atropine in *D. stramonium* extract, the level of this alkaloid was not changed in all experimental groups of the plants.

Table 3. The relative percentage of atropine and scopolamine in *D. stramonium* extract under the effect of SMF
Tabela 3. Zawartość względna atropiny i skopolaminy w ekstrakcie *D. stramonium* pod wpływem stałego pola magnetycznego

The studied factors Badane czynniki		Enzyme/ Enzym	
		the relative percentage of atropine relatywny odsetek atropiny (%)	the relative percentage of scopolamine relatywny odsetek skopolaminy (%)
Control/ Kontrola		3.5 ±0.21%	23.8 ±0.43%
30 min	3 mT	3.6 ±0.29%	20.7 ±0.31%*
	10 mT	3.4 ±0.13%	17.3 ±0.32% **
22 h	3 mT	3,5 ±0,13%	28.6 ±0.31%***
	10 mT	3,7 ±0,31%	21.7 ±0.34%

The statistical significance is *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared to the control
Istotność różnic przy * $P < 0,05$, ** $P < 0,01$, *** $P < 0,001$ w porównaniu z kontrolą

Table 4. Analysis of variance of SMF effect on level of TBA-active products, superoxide dismutase and catalase activity and the relative percentage of atropine and scopolamine
Tabela 4. Analiza wariancji wpływu stałego pola magnetycznego na poziom aktywnych produktów TBA, dysmutazę ponadtlenkową i aktywność katalazy oraz względny odsetek atropiny i skopolaminy

The studied factors Badane czynniki	Enzyme/ Enzym				
	TBA	catalase katalaza	superoxide dismutase dysmutaza ponadtlenkowa	atropine atropina	scopolamine skopolamina
Unmeasured factors Czynniki niemierzalne	16,5	29,7	36,8	77,2	0,8
Interaction / Interakcja	8,9***	21,3***	15,8***	16,6	16,3***
Intensity / Intensywność	2,9*	1,3	0,9	0,3	24,6**
Duration / Trwanie	71,7***	47,7***	46,5***	5,8	58,3***

The statistical significance is * P < 0.05, ** P < 0.01, *** P < 0.001, compared to the control
Istotność różnic przy * P < 0,05, ** P < 0,01, *** P < 0,001 w porównaniu z kontrolą

Thus, maximal effect of SMF on the relative percentage of scopolamine in the *D. stramonium* extract was observed at the influence of 3 mT with the duration of exposure of 22 hours. The SMF had no effect on the level of atropine. Alkaloids are a group of naturally chemical compounds that contain mostly basic nitrogen atoms [Iranbakhsh et al. 2006]. Perhaps, long-term effect of SMF on *D. stramonium* seeds leads to the violation of metabolic balance and nitrogen fixation in the cell plant.

The changes in the duration of exposure of the field caused the most severe effect on the studied parameters. This factor is the most significant for the level of TBA-active products, activity of superoxide dismutase and catalase and the relative percentage of scopolamine (Table 4). The particles of magnetic field influenced an interaction between two studied factors of antioxidant system. The duration of exposure of magnetic field was the most significant factor for seeds germination, the TBA-active products level, activity of superoxide dismutase and catalase and the amount of scopolamine in the extract.

CONCLUSIONS

Summarizing all the results of our research we can conclude that long-term effect of SMF on seeds (the influence of SMF of 3 mT and 10 mT with duration of exposure of 22 hours) leads to the increased level of TBA-active products, as well as increases activity of catalase and superoxide dismutase in *D. stramonium* seedlings in 1.31 and 4.8 times respectively. The results indicate the violations of the redox balance during of long-term SMF exposition in *D. stramonium* seeds. It causes, in turn, significant changes in the relative percentage of scopolamine in *D. stramonium* extract. A significant in-

crease of scopolamine was observed under the intensity of 3 mT and duration of exposure of 22 hours. The duration of exposure of magnetic field was the most significant factor that affected the TBA-active products level, activity of superoxide dismutase and catalase and the amount of scopolamine in the extract. The results indicate that the short-term effects of magnetic field exposition of 3 mT and 10 mT SMF intensity can be used as a regulator of growth processes in *D. stramonium.*

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Streszczenie. Działanie promieniowania magnetycznego na układ prooksydacyjny, antyoksydacyjny i zawartość alkaloidów w *Datura stramonium* L. (bieluń dzędzierzawa) dotychczas nie były badane. Celem niniejszej pracy było zbadanie działania stałego pola magnetycznego na homeostazę i zawartość alkaloidów w siewkach *Datura stramonium* L. W doświadczeniu przeanalizowano zawartość aktywnych produktów TBA (tiobarbitur kwas), dysmutazy ponadtlenkowej i aktywność katalazy w sadzonkach pod wpływem stałego natężenia pola magnetycznego 3 mT i 10 mT w czasie 30 minut lub 22 godzin. Zawartość alkaloidów w wyciągu z liści i łodyg siewek oznaczano metodą chromatograficzną. Długi czas oddziaływania stałego pola magnetycznego spowodował wzrost produktów peroksydacji lipidów, intensyfikację dysmutazy ponadtlenkowej i wzrost aktywności katalazy w siewkach. Zawartość skopolaminy zależy od czasu ekspozycji na działanie stałego pola magnetycznego, czynnik ten jednak nie miał istotnego wpływu na zawartość atropiny. Rezultaty badań zostały potwierdzone przez wyniki analizy wariancji.

Słowa kluczowe: *Datura stramonium*, stałe pole magnetyczne, peroksydacja lipidów, dysmutaza ponadtlenkowa, katalaza

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