The Dinitrophenol and Potassium Iodate Influence on *Hordeum Vulgare* Seedlings Viability

ELENA TODIRASCU CIORNEA¹, GABRIELA DUMITRU^{1*}, ION SANDU^{2,3}

¹ Alexandru Ioan Cuza University of Iasi, Faculty of Biology, Department of Biology, 11 Carol I Blvd., 700506, Iasi, Romania ² Alexandru Ioan Cuza University of Iasi, ARHEOINVEST Interdisciplinary Platform, 22 Carol I Blvd., 700506, Iasi, Romania ³ Romanian Investors Forum 2 Sf. Patru Mavila Str. Pl. 111 III/2, 700080, Iasi, Romania

³ Romanian Inventors Forum, 3 Sf. Petru Movila Str., Bl. L11, III/3, 700089, Iasi, Romania

The using of the pesticides of dinitrophenol type in agriculture has as consequence the major pollution of the environment, the plants taking these substances from the soil and once with these ones they reach in the human and animal organism where they product disequilibrium that are interpreted through the accumulation of free oxygen radicals with direct repercussions on the antioxidant enzyme's synthesis intensification and on their activity's increase. The apply of treatments on the barley seeds had significant effects regarding the seeds' germination, the young plants' growth, the oxidative stress enzymes' activity, but also regarding the content of photoassimilators and carotenoids pigments.

Keywords: dinitrophenol, KIO, oxidative stress, barley, germination

In the last years, were massively used in agriculture different organic and inorganic substances with the aim of obtaining risen agricultural production and of creating some varieties of high productivity, but also for the rebuttal of the diseases and of the pests [1]. The modern agriculture leads, but then, to the accentuated pollution of the environment, the negative strong impact being due to their long standing persistence in the soil and, from here on, implicitly, in the vegetable and animal organisms [2, 3]. Data from the specialty literature point out on the irrational using of the pesticides that product major disequilibrium at the flora's and fauna's level [4]. Although the dinitrophenol (DNP) and its derivates are toxic compounds with long standing remanence in the medium, these substances are still used on large scale in agriculture as pesticides, insecticides, ovicides, acaricides, fungicides, weed killer due to multiple biological actions that it manifests [5, 6] or even as the rapeutically agents [7, 8]. The plants, generally, and those ones superiors specially, are direct receptors of the agrotoxicity, being used as models for the monitoring of the negative impact of the pesticides [9].

Being given the fact that the *Hordeum vulgare* species is, on the one hand a grass tenacious to different stress conditions [10], and on the other hand that it has a big importance for the human's and animals' food, being significantly reach in bioactive compounds like â-glucan, tocols and polyphenolic compounds [11], the present work had as aim the testing of seedling barley viability under the direct influence of DNP and KIO₃ in different concentrations.

Experimental part

Equipment, materials and methods Biological material

Were taken into study the *Hordeum vulgare* ssp. *vulgare* seeds from the harvest of 2015, derived from S.C.D.A Suceava.

Reagents and instruments

All the reagents used were of high analytic purity and derived from Sigma-Aldrich and Merck. The solutions were prepared in distillated water obtained in a MilliQ (18,2 U) substance, and the UV-VIS measurements were realized with a *Shimadzu UV-VIS 1700* spectrophotometer, in quartz vats of 1 cm towards a control of the reagents.

Protocols

The seeds were treated previously, for 2 respectively 4 hours, with solutions of 2,4 -DNP and potassium iodate (KIO₃) in concentrations of 10^3 , 10^4 and 10^5 M, in parallel with soaked samples in water. For each type of treatment in part, were done three parallel determinations, after the saturation the seeds being layed out in Petri boxes. For 10 days the samples were wetted with a constant volume of water, and after the seedlings harvest were evaluated the germinative faculty of caryopses, the height and the weight seedlings, as well as the oxidative stress enzymes' activity, the total soluble proteins concentration and the content of assimilators pigments.

The activity of superoxide-dismutase (SOD, 1.15.1.1) was evaluated by the Winterbourne method [12], which is based on the ability of the enzyme to inhibit the reduction of Nitro Blue Tetrazolium by superoxide radicals generated in the reaction medium by the riboflavin's fotoreduction, the inhibition degree product by the enzyme, under standard conditions, being estimated through determining the extinctions at a wavelength of 560 nm.

The dosage of the catalase activity (CAT, EC 1.11.1.6) was made through the Sinha method, which is based on the colorimetric determination ($\lambda = 570$ nm) of the chromic acetate obtained through the reduction reaction of the potassium dichromate in an acidic medium by decomposing the remaining hydrogen peroxide after the inactivation of the enzyme [13].

The peroxidase (PÓX, EC 1.11.1.X.) was determined through the Gudkova and Degtiari method with odianisidine, the formed colored product having a maximum absorption at 540 nm [12].

For calculation the specific activity, which renders as closely as possible, the real catalytic capacity of the enzymes, we determined the concentration of the total soluble protein using the Bradford method which is based on the observation that, in an acid medium, the dye Coomassie Brilliant Blue G-250 forms with the proteins a complex with maximum absorption at 595 nm [13].

^{*} email: email: gabriela.dumitru@uaic.ro

The chlorophyll and carotenoid pigments content was estimated spectrophotometrically after the extraction with acetone at wavelengths of 663, 645 and 472 nm [14].

Statistics

The obtained experimental results were statistically analyzed using SPSS20 program and Microsoft Excel (the Student t-test), being considered significant at *p* values of less than 0.05.

Results and discussions

Taking into account the fact that, in the last years, have been used in increasing amounts pesticides in the agriculture, even if the harmful effect thereof on the human and animal organisms is well known, in a first series of experiments we used the determination of the germinative faculty of *Hordeum vulgare* seeds treated with DNP (an agent which is part of many pesticides and which has a very high toxicity) and KIO₃ in different concentrations (10³, 10⁴ and 10⁵M).

If at the control group (table 1) the germinative faculty has reached values of $94.66 \pm 1.013\%$ in the case of the seeds that were soaked for two hours, respectively $95.633 \pm 0.817\%$ at those soaked for four hours, the DNP treatment determined radically the reducing of the germination rate. Thus, at the plots immersed for two hours, the germination degree of the barley seeds varied between $66.166 \pm 3.113\%$ at 10^{-3} M concentration solution, and $89.166 \pm 3.655\%$ at the concentration of 10^{-5} M, while, the extension treatment (4 h of soaking), leaded to a germination rate of only 27.666 $\pm 0.817\%$ (10^{-3} M solution).

Our data chime in with the specialty literature which indicates the fact that the treatments with pesticides reduce the germination degree of barley seeds [15, 16], the pesticides penetrating inside the seeds by diffusion, where there is the synthesis inhibition or a hydrolytic enzymes' activity [17], being affected by vital enzymes such as amylase, ATP-ase, lipase and protease and thus essential metabolic pathways mediated by those ones [18].

By comparison, KIO₃ hadn't such a marked influence on the degree of germination (table 2), and may refer, however, a slight decrease in the case of the two hours treatment (83.333% for 10^3 and 10^5 solutions and 86.166 \pm 5.811% in the case of 10^4 M) but also a minor stimulation to the samples for four hours immersed in the treatment solutions (97.166 \pm 0.881% in the case of KIO³ 10^{-5} solution).

The influence of the two types of treatment can be correlated with other data from the specialty literature indicating that substances such dinitrophenol can be inhibitors consecrated of the various metabolic processes [19].

As for the weight barley seedlings (tables 3-4), the samples treated with DNP, it's ascertained the same trend of decreasing value to control, closely related to the concentration degree of the respective agent. If at two hours of soaking, the weight of the seedlings is almost identical to that decelated in the control, at four hours of treatment, the weight of seedlings reached the threshold of only 0.055 ± 0.006 g/seedling in 10^{-3M} solution, 0.065 ± 0.003 g/seedling at that of 10^{-4} M and 0.072 ± 0.001 g/seedling to 10^{-5} M, comparatively to the reference, in which the weight of the seedling has reached 0.081 ± 0.0009 g.

In comparison, the apply of KIO₃ treatment induced different responses, in the sense that, at the 10^{-4} M concentration (2 h of treatment) the seedlings had a lower weight than the controls (0.079 ± 0.003 g /seedling

 Table 1

 GERMINATIVE FACULTY (%) OF BARLEY SEEDLINGS PREVIOUSLY

 TREATED WITH DNP AND ITS MAIN STATISTICAL INDICES

		Control	DNP		
			10-3M	10-4M	10-°M
	М	94.66	66.166	83.833	89.166
	s x	1.013	3.113	2.315	3.655
2	S (σ)	1.755	5.392	4.01	6.331
hou	Conf.	4.362	13.396	9.962	15.727
1	SL	99.028	79.563	93.795	104.894
	IL	90.304	52.769	73.87	73.439
	VC%	1.854	8.15	4.783	7.1
	m%	1.071	4.70	2.761	4.099
	М	95.633	27.666	73.8	84.5
	s x	0.817	2.185	4.403	1.802
	S (σ)	1.415	3.785	7.626	3.122
4 hours	Conf.	3.516	9.404	18.946	7.756
	SL	99.149	37.071	92.746	92.256
	IL	92.117	18.261	54.853	76.743
	VC%	1.48	13.684	10.334	3.695
	m%	0.854	7.9	5.966	2.133

M = mean, S χ = standard error, S (σ) = standard deviation, Conf. = confidence level; SL = superior limit, IL = inferior limit, VC% = average variation coefficient; m% = average precision coefficient

 Table 2

 GERMINATIVE FACULTY (%) OF BARLEY SEEDLINGS PREVIOUSLY

 TREATED WITH KIO, AND ITS MAIN STATISTICAL INDICES

		Control	KIO3			
		Control	10-3M	10-4M	10-°M	
	М	94.66	83.333	86.166	83.333	
	sx	1.013	3.632	5.811	3.004	
1.5	S (σ)	1.755	6.291	10.066	5.204	
hou	Conf.	4.362	15.629	25.004	12.927	
2	SL	99.028	98.962	111.173	101.261	
	IL	90.304	67.704	61.16	75.405	
	VC%	1.854	7.549	11.682	5.891	
	m%	1.071	4.358	6.744	3.401	
	М	95.633	87.666	91.166	97.166	
	sx	0.817	2.242	2.333	0.881	
	S (o)	1.415	3.883	4.041	1.527	
sino	Conf.	3.516	9.647	10.039	3.794	
4 hc	SL	99.149	97.314	101.206	100.961	
	IL	92.117	78.018	81.127	93.372	
	VC%	1.48	4.43	4.433	1.572	
	m%	0.854	2.557	2.559	0.907	

 $M = mean, S = standard error, S (\sigma) = standard deviation, Conf. = confidence level; SL = superior limit, IL = inferior limit, VC% = average variation coefficient; m% = average precision coefficient$

comparatively with 0.082 \pm 0.001 g/seedling), and at the 10 5 M (4 h of treatment) slightly higher (0.0845 \pm 0.003 g /seedling, respectively 0.081 \pm 0.0009 g /seedlings).

			DNP		
		Control	10-3M	10-	10-
				4M	⁵M
	М	0.082	0.074	0.081	0.083
	s x	0.001	0.0008	0.002	0.001
sino	S (o)	0.002	0.001	0.004	0.003
2 h	Conf.	0.007	0.003	0.012	0.007
	SL	0.089	0.077	0.093	0.09
	IL	0.075	0.07	0.069	0.075
	VC%	3.508	1.958	5.999	3.666
	m%	2.025	1.131	3.463	2.116
	М	0.0810	0.055	0.065	0.072
	s x	0.0009	0.006	0.003	0.001
	S (σ)	0.001	0.011	0.005	0.003
4 hours	Conf.	0.004	0.027	0.014	0.007
	SL	0.086	0.082	0.079	0.08
	IL	0.077	0.028	0.05	0.065
	VC%	2.078	19.799	9.087	4.165
	m%	1.199	11.431	5.246	2.405

 Table 3

 DNP INFLUENCE ON BARLEY SEEDLING WEIGHT (g)

 AND ITS MAIN STATISTICAL INDICES

 $M = mean, S = standard error, S (\sigma) = standard deviation, Conf. = confidence level; SL = superior limit, IL = inferior limit, VC% = average variation coefficient; m% = average precision coefficient$

 Table 4

 KIO3 INFLUENCE ON BARLEY SEEDLING WEIGHT (g) AND ITS MAIN STATISTICAL INDICES

			KIO3		
		Control	10-	10-⁴M	10∹M
			${}^{3}\mathbf{M}$		
	М	0.082	0.086	0.079	0.083
	sx	0.001	0.001	0.003	0.001
ours	S (σ)	0.002	0.002	0.005	0.002
2 h	Conf.	0.007	0.007	0.013	0.005
	SL	0.089	0.093	0.092	0.088
	IL	0.075	0.079	0.065	0.078
	VC%	3.508	3.285	6.806	2.58
	m%	2.025	1.896	3.929	1.489
	М	0.0810	0.077	0.0805	0.0845
	sx	0.0009	0.001	0.003	0.003
	S (σ)	0.001	0.001	0.006	0.005
4 hours	Conf.	0.004	0.004	0.015	0.013
	SL	0.086	0.082	0.096	0.098
	IL	0.077	0.072	0.064	0.07
	VC%	2.078	2.531	7.852	6.627
	m%	1.199	1.461	4.533	3.826

 $M = mean, S = standard error, S (\sigma) = standard deviation, Conf. = confidence level; SL = superior limit, IL = inferior limit, VC% = average variation coefficient; m% = average precision coefficient 2162 http://www.r$

The specialty literature indicates [20, 21], moreover, the appearance of some important changes in the normal development of the seed which is in the process of germination, closely related to the type of substances, their concentration and their toxicity.

If at the control group, the seedlings have reached the height of 10.773 ± 0.138 cm (at 4 h of soaking) and 10.977 ± 1.109 cm (at 2 h), in the case of the DNP there is an inhibition of the elongation process of the seedlings in close correlation with their weight and the percentage of germination of the seeds, at 4 h after treatment the average height of the barley seedlings ranging from 6.997 ± 0.218 cm - 10^{-3} M solution, and 9.42 ± 0.077 cm - at 10^{-5} M solution (table 5).

The treatment with KIO_3 applied to barley caryopses (table 6), had a slightly unexpected effect, in the sense that the seedlings which were plated lower weights recorded an inverse relationship to their height and vice versa (the higher seedlings were thinner and, automatically, lighter and those shorter ones had higher weight). At the same time, we may emphasize a higher size of the seedlings grown from seeds treated for 2 h with KIO₃ 10³M (12.19±0.41cm) comparatively to the group obtained after 4 h of immersion solution KIO₃ 10⁴ M (9.913±0.245 cm).

 Table 5

 DNP INFLUENCE ON BARLEY SEEDLING HEIGHT (cm) AND ITS

 MAIN STATISTICAL INDICES

		Control	DNP		
		Control	10-3M	10-4M	10-°M
	М	10.977	9.946	10.55	11.09
	sx	1.109	0.568	0.088	0.1
13	S (o)	1.921	0.985	0.153	0.174
hou	Conf.	4.772	2.447	0.382	0.433
5	SL	15.749	12.394	10.932	11.523
	IL	6.204	7.499	10.167	10.656
	VC%	17.501	9.904	1.459	1.572
	m%	10.104	5.718	0.842	0.907
	М	10.773	6.997	8.56	9.42
	sx	0.138	0.218	0.234	0.077
	S (σ)	0.24	0.378	0.402	0.134
4 hours	Conf.	0.596	0.941	1.001	0.334
	SL	11.37	7.938	9.56	9.754
	IL	10.176	6.055	7.559	9.085
	VC%	2.23	5.415	4.703	1.428
	m%	1.287	3.126	2.715	0.824

 $M = mean, S = standard error, S (\sigma) = standard deviation, Conf. = confidence level; SL = superior limit, IL = inferior limit, VC% = average variation coefficient; m% = average precision coefficient$

Data from the specialty literature signalize the fact that a series of substances such as DNP produce important metabolic disturbances at the plants' metabolism level with direct effects on these ones, due to generating the oxygen reactive species (ROS), the oxidative stress producing a perturbation of the equilibrium between the pro-oxidative reactions and those antioxidant [21]. Given the fact that, the strong negative impact of the oxidative stress on different tissues and organs, the plants created themself different defense strategies, in these ones a

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		Control	KIO3		
			10-3M	10-4M	10-°M
	М	10.977	12.19	11.563	11.853
	sx	1.109	0.41	0.288	0.098
rs	S (σ)	1.921	0.711	0.5	0.17
hou	Conf.	4.772	1.767	1.243	0.423
6	SL	15.749	13.957	12.806	12.276
	IL	6.204	10.422	10.319	11.43
	VC%	17.501	5.835	4.328	1.437
	m%	10.104	3.369	2.499	0.829
	М	10.773	11.916	9.913	9.31
	sx	0.138	0.311	0.245	0.157
	S (σ)	0.24	0.54	0.425	0.272
sino	Conf.	0.596	1.341	1.056	0.676
4 h	SL	11.37	13.258	10.97	9.986
	IL	10.176	10.575	8.856	8.633
	VC%	2.23	4.531	4.291	2.923
	m%	1.287	2.616	2.477	1.688

 Table 6

 KIO3 INFLUENCE ON BARLEY SEEDLING HEIGHT (cm) AND ITS

 MAIN STATISTICAL INDICES

 $M = mean, S = standard error, S (\sigma) = standard deviation, Conf. = confidence level; SL = superior limit, IL = inferior limit, VC% = average variation coefficient; m% = average precision coefficient$

special apart being occupied by the enzymatic antioxidant system [22, 23]. Therefore, another objective of our study it was the SOD activity determination - as a first barrier in the defense against ROS, this metalo-enzyme interfering in the converting of the superoxide radicals generated after the biological oxidation processes.

At the cellular level, SOD is the first line of defense against reactive oxygen species defense, the enzyme converting the superoxide radicals into oxygen and hydrogen peroxide [24, 25]. This metallo-enzyme is found in all aerobic organisms where an important role plays in the defense against ROS generated as the main products of biological oxidation [26, 27]. Applying the above mentioned treatments had as repercussions the growth as more or less significant of the SOD activity regardless the time of the seeds' exposure to the action of the agents



Fig.1. The SOD activity in *Hordeum vulgare* seedlings previously treated with DNP and KIO₂ of different concentrations

 $\label{eq:product} \begin{array}{l} (*p < 0.05 - less \ significant; **p < 0.01 - significant; ***p < 0.001 - \\ very \ significant) \end{array}$

used. Thus, at two hours of soaking the SOD activity (fig. 1) varied between 10.199 ± 0.797 SODU/µg protein at the reference variant, 12.216, respectively 12.306 SODU/µg protein for treatments with DNP 10⁻⁴ and 10⁻⁵M for DNP 10⁻³M solution to produce the most significant stress, translated into a activity value level of 14.848±1.557 SODU/ µg protein. The longer period of exposure of the Hordeum vulgare L. ssp. vulgare caryopses at soaking with DNP and KIO₃ solution remarked itself through significant enzymatic activities (p < 0.01) and strongly significant (p < 0.001) comparatively with the reference. For example, the DNP 10⁻³M solution induced a level of stress layed out through values of activity such as 16.321 ± 0.632 SODU/µg protein, while the KIO_3^{3} 10³ and 10⁴M solutions determined an activity of this oxidoreductase of 15.868±0.943 SODU/µg protein, respectively 15.649 ± 0.902 SODU/µg protein.

In explaining the results we must take into account the fact that a particularly important factor in evaluating the oxidoreductases' activity is the period that elapses from the moment of applying the treatment and the effective practical determination [28], knowing the fact that the SOD activity intensify itself during the radiculs' emergence (at 24-48 h from soaking), this increase being more significant in embryo than in endosperm, as more as the seedlings elongate the activity of this enzyme increases, but with a reduced speed [29-31].

The intensive using in agriculture of the different pesticides, determines a massive accumulation of these ones in the superficial or deeper stratum of the soil and, from here on, there are easily absorbed by the radicular systems of the plants, being accumulated at the tissues' and organs' level [32-37]. This thing has as result the increase of the oxidative stress level and, implicitly, of the antioxidant enzymes' activity, CAT being a key barrier against the free radicals through transforming these ones in non-toxic products [38, 39].

The analyze of our results regarding the influence that exerts the DNP, which is in different concentrations, on the CAT activity in the seedlings barley of ten days show differences strongly significant between the control plots and those treated (fig. 2). Thus, at two hours of immersion, the enzymatic activity reached the valoric level of 22.191 \pm 1.555 CATU/µg protein in the case of the reference sample and between 34.227 \pm 2.021 CATU/µg protein and 44.142 \pm 2.031 CATU/µg protein in the case of those treated with DNP (10⁻⁵M and 10⁻³M), while, after applying the treatment of four hours, the activity stayed almost unmodified in the control (23.016 \pm 1.142 CATU/µg protein) for as in the DNP 10⁻³M solution's case, for example, to



Fig.2. The CAT activity in *Hordeum vulgare* seedlings previously treated with DNP and KIO_3 of different concentrations (*p < 0.05 - less significant;**p < 0.01 - significant; **p < 0.001 - very significant)

reach a level approximately three times higher $(61.863\pm2.541 \text{ CATU/}\mu\text{g} \text{ protein})$. In what concerns KIO_3 we can observe an influence a bitter significant in the case of seeds soaking for 4 h, the enzymatic decelated activity reaching valoric thresholds of $36.278\pm0.781 \text{ CATU/}\mu\text{g}$ protein at the 10^{-4}M solution, respectively $39.526\pm1.146 \text{ CATU/}\mu\text{g}$ protein at that of 10^{-3}M concentration.

Our results could be explain through that, in concordance with the specialty literature, during the germination of *Hordeum vulgare* seeds, the aleurone layer cells synthesis and secrete hydrolytic enzymes, and the gluconeogenese of lipids' reserves from the aleurone cells, which stands this synthesis of hydrolytic enzymes, has as result the H_2O_2 generation which is catabolized by the glyoxysomal catalase [40]. On the other hand, the accumulation of antioxidant enzymes has a significant effect in the conditions in which the biotic or abiotic stress instals itself mainly in the early stage of growing and development of the seedlings barley [10, 41] a significant importance having, so, the age of seedlings and the moment of applying treatments.

The statistical analyze confirms a remarkable influence of the DNP treatment, especially in the concentrations of $10^{-3} - 10^{-4}$ M observing strongly significant differences (*p*<0.001) by comparison with the reference plot both at two, as at 4 h treatment, being knew the strongly nocive effect of this agent on plants by the appearance of some major imbalances of the metabolic reactions [4].

The next stage of our study was to determine the POX activity in the barley seedlings bended to the two types of treatment, enzyme with significant role in the oxidation process and the axis growth of the seeds bended to germination [42, 43], but also in the layer cells metabolism, lignification and suberization, the resistance of the plants at the action of different xenobiotics, the auxines' metabolism, the cells elongation, the fenolic oxidation etc. [44-46].

If in the case of the control plot, the immersion period of the caryopses before put to germination (fig. 3), hadn't a decelable effect (15.223 ± 0.837 POXU/µg protein at two hours of soaking 15.525±1.233 POXU/µg protein at 4 hours), in the case of the variants treated with DNP and KIO₃ were observed some differences, in the sense that, the DNP 10⁻³M solution induced a significant oxidative stress (p < 0.01) and more significant (p < 0.001), the peroxidasic activity reaching values of 26.447±1.499 POXU/µg protein at the shorter period of soaking and 30.547±1.445 POXU/ ig protein in the case of exposing for 4 h. In the case of KIO, solution the situation was slightly different, at the concentration of 10⁻³ and 10⁻⁵M the activity being increased less significant towards the control sample (16.889 ± 1.517) POXU/µg protein and 17.556±0.993 POXU/µg protein at the 10^{-5} M solution, respectively 18.789±1.895 POXU/µg protein and 20.131±1.181 POXU/µg protein in the case of 10⁻³M concentration). The strongest effect had this agent, in concentration of 10^{4} M (p<0.01), at 4 h of soaking maying decelate an activity of this haemoprotein equal with 24.617 ± 0.467 POXU/µg protein.

It is known the fact that pollutants exert on plants fitotoxic effects with consequences mostly in what concerns the biochemic, the physiologic, the morphologic and the citogenetic transformations [47-51], the photosynthese being one of the processes that has a lot to suffer because of the action of different biotic and abiotic factors.

In what concerns the chlorophyll **a** content in the samples analyzed by us, we can pin down a variation more or less significant in function of the type of treatment (fig .4). Thus, the DNP solutions had an inhibitor effect on 2164 http://www.rd



Fig.3. The POX activity in *Hordeum vulgare* seedlings previously treated with DNP and KIO₃ of different concentrations

(*p < 0.05 – less significant; **p < 0.01 – significant; ***p < 0.001 – very significant)

the synthesis of chlorophyll **a**, the valoric levels decelated being of approximately 0.2 mg/g fresh tissue (10^{-3} M) and 0.25 mg/g fresh tissue (concentrations of 10^{-4} and 10^{-5} M) comparatively with those decelated in the control plot control (0.277±0.03 mg/g fresh tissue in the case of soaking during 2 h and 0.285±0.025 mg/g fresh tissue at 4 h of exposing).

These data are also sustained by the specialty literature which signalizes the fact that the pesticides reduce the level of chlorophylien pigments, strongly related to the degree of concentration of the agent used, with direct implication on the foliar surface and, implicitly, on the photosynthetic apparatus [17].

In the case of applying the treatment with KIO₃ it is remarked a different situation, in the sense that the variants treated with 10^4 and 10^5 M solution got to the accumulation of some enhanced quantity of this photoassimilator pigment, both at 2 as at 4 h of soaking (0.38, respectively 0.34 mg/g fresh tissue). The stimulating effect of KIO₃ is also validated by the applying of the Student test of statistical signification which shows the existence of some significant and strong significant differences comparatively with the control plot.



Fig.4. The content of chlorophyll *a* in *Hordeum vulgare* seedlings previously treated with DNP and KIO₃ of different concentrations (*p < 0.05 . less significant;**p < 0.01 . significant; ***p < 0.001 . very significant)

The chlorophyll **b** is an accessory pigment of the systems that need light for growing like superior plants, the green algae, *Euglenaceae* and *Prochlorophyta* being able to reach up to 30% from the total assimilatory pigments [52], the vegetal organisms possessing two photosystems that pull together to realize the photosynthesis process [53].

Unlike the chlorophyll a content, the applying of treatments with DNP and KIO₃ had contradictory results

regarding the chlorophyll **b** (fig. 5), in the sense that, unlike the reference plot, the different concentrations of treatment exerted inhibitory or stimulatory effects on the valoric level of this pigment. Thus, the 10^{-4} M solutions stimulated the synthesis and the accumulation of this type of chlorophyll (0.114±0.009 mg/g fresh tissue in DNP case and 0.121±0.01 mg/g fresh tissue for KIO₃), while the control layed out a medium content per plot of approximately 0.08 mg/g fresh tissue.



Fig.5. The content of chlorophyll *b* in *Hordeum vulgare* seedlings previously treated with DNP and KIO₃ of different concentrations (*p < 0.05 . less significant;**p < 0.01 . significant; ***p < 0.001 . very significant)



Fig.6. The content of carotens in *Hordeum vulgare* seedlings previously treated with DNP and KIO₃ of different concentrations (*p < 0.05 . less significant;**p < 0.01 . significant; ***p < 0.001 . very significant)

Data from the specialty literature signalize that the carotenoids pigments are the most important photosynthetic pigments, with role to protect the tilacoids membranes and chlorophylls [54], results obtained in the case of our samples (fig. 6) highlighting significant and strongly significant differences between the reference plots (0.06 mg/g fresh tissue) and the variants treated with DNP and KIO₃ 10^{-4} and 10^{-5} M which oscillate between 0.052 ± 0.002 mg/g fresh tissue (DNP 10^{-5} M - 2 h treatment) and 0.082 ± 0.002 mg/g fresh tissue (KIO₃ 10^{-4} M - 4 h of treatment).

Conclusions

The obtained results demonstrate the high toxicity of the DNP on the *Hordeum vulgare* L. seedlings viability being affected in a large measure the germinative faculty, the weight and the plants high. In the same time, the applying of the treatments induced the apparition of some strong oxidative stress, translated by an accumulation of some enhanced quantity of free radicals which determined markant increasing of the SOD, CAT and POX activity, but also the synthesis' inhibition of chlorophyliens and carotenoids pigments, the time of exposure at treatment being a decisive factor in this sense.

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