The Influence of Dinitrophenolic Pesticides on the Viability of Plants

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Biodiversity conservation on global plan, but in our country as well is an important research area that is necessary for understanding the characteristics of ecological and social agrobiodiversity and acknowledging the benefits brought for ecosystem and society. Currently the increase of agricultural production is obtained by using pesticides and insecticides, fossil fuels, irrigations, and by using modern soils with a great productivity. Even if the agricultural methods mentioned above can offer a simplified and easy management, on long term this kind of agriculture can cause the environment pollution, having drastic consequences on the human health and the irreversible loss of agricultural diversity. In consequence, this study investigates the effect of dinitrophenolic pesticides on the viability of plants demonstrated by germination studies.

Keywords: dinitrophenolic pesticides; biodiversity; conservation; pollution; germination tests

Agrobiodiversity represents a branch of biodiversity, that is based on the variety and variability study of organisms such as microorganisms, plants and animals important in agriculture, and whose existences is influenced by three main factors such as: the interaction with the environment, genetic resources and the management of anthropic agricultural system [1-3]. The anthropic activity can have an harmful effect on biodiversity by reducing many species [4]. It is estimated that during a lifetime, man used approximately 10.000 species of crop plants; nowadays, according to the FAO (Food and Agriculture Organization) statistics, approximately 90% of food production is provided by around 120 species of crop plants (FAO, 2014). The appearance and development of industrial agriculture led to the process of genetic erosion, and determined the replacement of old soils and local variety of crop plants with more modern and profitable ones [5, 6].

Because of the increase with approximately 60 million annually of the global population, the increase of the agricultural production becomes an extremely important global objective, an increase that can be realized also by combating pests. The substances that exert a toxic effect on the pests that cause significant loss in different sectors of human activity are known as pesticides [7]. Bacteria, fungi, parasites, insects, mites, rodents, nematodes, etc. are examples from different groups of pests that can endanger the humans' and animals' health or that can decrease the quality of environment [8].

The complexity of this research area requires extensive studies, regarding the structure and activity of pesticides, the correlation between the mechanism of action and chemical structure, the toxicity on humans and animals, and the influence on the environment [9-11]. This applied science, called also the pesticide science, is a borderline science between biology, organic chemistry and biochemistry, and applied sciences of the agricultural sector as well [12].

During time, especially the urban population searched for the most efficient production methods, and tried the selection and enrichment of local traditional varieties, without disturbing the soil and natural laws of the environment [13]. In old times, there wasn't the problem of protection the genetic resources and of forming long term agriculture, a problem that nowadays is acknowledged due to the population awareness that the extinction of these seeds and the soil alteration could lead to the civilization death [14].

The irrational use of pesticides can cause the extinct of some plant species and in consequence the disorder of the equilibrium between flora and fauna. The degradation products resulted in time present different degrees of danger for the environment, flora and fauna, depending on their own structural characteristics or of the initial compound, remanence in body being about 1 month and half [15]. Dinitrophenols are extremely toxic compounds, they are difficultly eliminated from organism, and their lifetime in the environment is pretty long of about 4 months. Nevertheless, the action of some water microorganisms can be an accessible method for the degradation of dinitroderivatives [16, 17]. Even if these agricultural methods can offer a simplified and easy management, on long term this kind of agriculture can cause the environment pollution, having drastic consequences on the human health and the irreversible loss of agricultural diversity [18].

Dinitroderivatives, especially the aromatic ones, are compounds with multiple biological activities and are used as insecticides, ovicidals, acaricides, fungicides and herbicides and they play an important role in chemical protection of plants [19]. This group of herbicides exert contact action [20]. Their conditioning, namely the way they are used in current practice, can be realized in different forms such as: emulsifiable concentrates, mineral oil solutions, soluble salts, wettable powders that are applied especially for cereal and legume crops [21]. The pesticides

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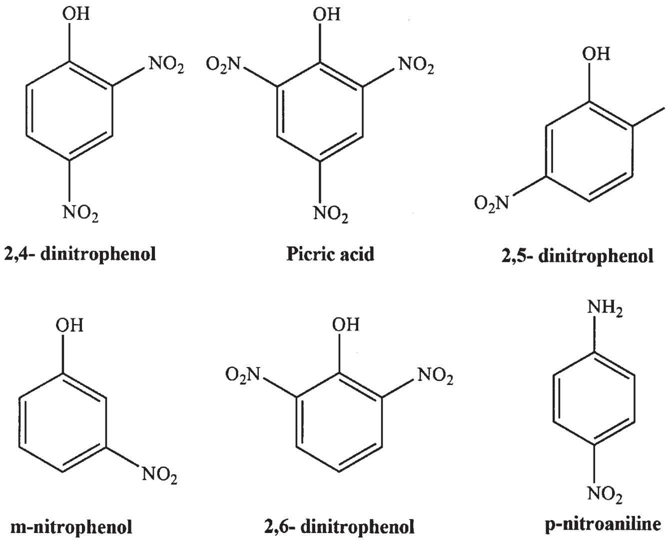

Fig. 1. Chemical structure of the investigated dinitrophenolic pesticides: 2,4-DNP (2,4-dinitrophenol), picric acid, 2,5-DNP (2,5-dinitrophenol), 2,6-DNP (2,6-dinitrophenol), p-nitroaniline

selection for practical applications implies the compliance of some basic requiements regarding the actions against the pests and the toxic effect for humans and environment [22].

Our main goal for this paper was to study the viability of plants that can be partially and reversible degraded in a specific way under the action of dinitrophenolic pesticides. In our studies we realised germination tests on plants in presence of nitrophenolic pesticides (phenolic compounds). The viability of plants was investigated by means of UV-Vis spectroscopy and by germination tests led in the presence of different categories of dinitrophenolic pesticides.

Experimental part

Materials and Methods

Reagents. The used reagents were of analytical grade, and the aqueous solutions were prepared with double distilled high purity water (R = 18.2 Ω). Dinitrophenols were acquired from Sigma-Aldrich (SUA).

Biological materials. Wheat seeds *Triticum aestivum*, variety Gasprom, were acquired from Agricultural Research Station, Suceava, Romania.

Instruments. Spectrophotometer UV-Vis model Libbra S35 PC UV/VIS ((Biochrom, UK) equipped with quartz cuvettes with optical length of 1 cm [23-25]. Analytical balance Vibra HT-224 CE (Shinko Denshi – Japonia), mass accuracy of 0.0001 g.

Protocols. The germination tests were conducted according to ISTA (Seed Science and Technology, 1993).. Batches of 50 seeds each were treated with different dinitrophenols such as: 2,4-dinitrophenol; 2,5-dinitrophenol; 2,6-dinitrophenol; picric acid; m-nitrophenol and p-nitroaniline (fig. 1), then placed on filter papers in sterile Petri dishes and left for germination. The germination tests were realised in triplicates. The seeds were treated with he mentioned pesticides for an hour; afterwards the seeds were evenly distributed on filter paper in sterile Petri dishes. 5 mL of double distilled water were daily added to the seeds. Seedlings, germinated seeds and dead seeds were counted after 7 days. The plants were cut from seeds, weighted (mass in grams), and measured (height in centimetres) [26-30].

The UV-Vis spectra of pure 2,4-dinitrophenol and of 2,4-dinitrophenol from the treatment solution applied to a standard batch of 50 seeds was recorded in the wavelength domain of 200-700 nm.

Absorption of the treatment solution by wheat seeds. 50 wheat seeds are selected, weighted at the analytical balance having initial mass m_i and added in a tube. A fresh prepared solution 2 x 10^{-3}M of 2,4-dinitrophenol is added to the tube containing the seeds and the tube is stirred from 5 to 5 min during an hour. The tube is left at room temperature for 24 h to allow the absorption of 2,4-dinitrophenol solution by the wheat seeds. After 24 h, the 2,4-dinitrophenol solution is filtered, the wheat seeds are dried in order to remove the solution from the seeds and then weighted having the finale mass after the treatment m_f.

Results and discussions

In this study was investigated the absorption and distribution of the nitroderivatives in seeds and it was noticed that it is different in case of nitrophenolderivatives.

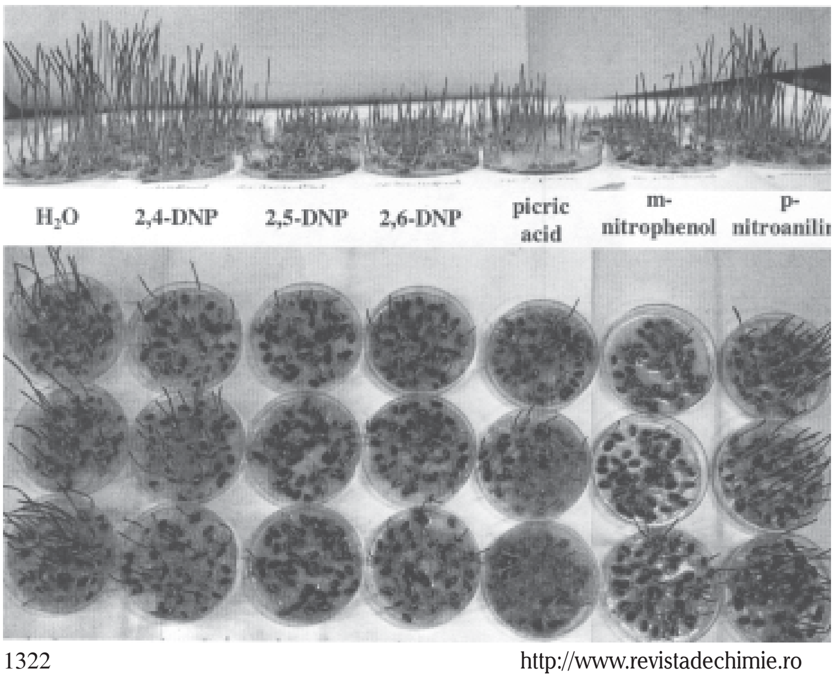

Fig. 2. The effect of different aromatic derivatives on the germination of the wheat seeds

	Seedlings	Germinated seeds	Dead seeds	Seedlings weight (g)	Seedlings height (cm)	Unit mass (mg)	Average height (cm)
Control distilled water	37	3	10	1.581	241.3	42.72	6.79
	33	2	15	1.468	233.6	44.48	7.07
	30	4	16	1.352	214.9	45.06	7.16
Average	**33.3**	**3**	**13.6**	**1.467**	**229.93**	**44.08**	**7**
2.4-DNP	28	2	20	1.341	180.5	47.89	6.44
	36	1	13	1.002	132.1	27.83	3.66
	26	1	23	0.911	130.7	35.03	5.02
Average	**30**	**1.3**	**18.6**	**1.084**	**147.76**	**36.91**	**5.04**
2.5-DNP	27	2	21	0.43	28.1	15.92	1.04
	30	3	17	0.694	45.2	23.13	1.5
	30	0	20	0.608	39.7	20.26	1.32
Average	**29**	**1.6**	**19.3**	**0.577**	**37.66**	**19.77**	**1.28**
2.6-DNP	32	1	17	0.813	87.8	25.4	2.74
	24	1	25	0.511	62.7	21.29	2.61
	25	1	24	0.582	66.3	23.28	2.65
Average	**27**	**1**	**22**	**0.635**	**72.26**	**23.32**	**2.66**
Picric acid	32	1	17	0.862	93.7	26.93	2.92
	24	4	22	0.725	71.2	29.6	2.96
	21	4	25	0.659	60.9	31.38	2.9
Average	**25.6**	**3**	**21.3**	**0.748**	**75.26**	**29.3**	**2.92**
m-nitrophenol	32	1	17	0.856	121.8	26.75	3.8
	28	2	20	0.355	59.4	12.67	2.12
	25	2	23	0.33	57.2	13.2	2.28
Average	**28.3**	**1.6**	**20**	**0.513**	**79.46**	**17.54**	**2.73**
p-nitroaniline	30	3	17	2.483	154.3	82.76	5.14
	32	2	16	2.607	165.1	81.46	5.18
	30	3	17	2.471	151.1	82.36	5
Average	30.6	2.6	16.6	2.52	156.8	82.19	5.1

Table 1
THE EFFECT OF DIFFERENT NITROAROMATIC DERIVATIVES ON THE GERMINATION OF THE WHEAT SEEDS

In figure 2 can be noticed that after removing the unabsorbed toxic, the seeds left to germinate in Petri dishes presented different growths. Also, it was observed that after 1 hour of treatment, the toxics penetrated the cells due to their high concentration ($2x10^{-3}M$). If the seeds are maintained for a long time in the treatment solution containing toxic compounds, the plants are progressively affected and can absorb a new quantity of toxics.

In table 1 are presented experimental data gathered after 7 days of treatment. From the toxicity point of view, it can be observed that the picric acid inhibits the most the development of the wheat seeds.

In accordance with the number of grown seedlings, the toxicity of compounds increases in the following order: p-nitroaniline <2,4-dinitrophenol (2,4-DNP) <2,5-dinitrophenol (2,5-DNP) < m-nitrophenol <2,6-dinitrophenol (2,6-DNP) < acid picric. In accordance with the height of the seedlings, the toxicity of the compounds increases in the following order: p-nitroalinine < 2,4-DNP < picric acid < m-nitrophenol < 2,6-DNP < 2,5-DNP, from these data one can conclude that 2,5-dinitrophenol has a major influence on the seedlings growth. After intoxication with 2,5-DNP, it was obtained an average height of seedlings of 1.28 cm, 2 times smaller than the height obtained for the seedlings that grew from seeds treated with 2,6-DNP (the next compound in the toxicity order) and only a quarter from the height of the seedlings intoxicated with p-nitroaniline, the last one being considered to have less toxic effects on the viability of wheat seedlings.

Taking into account the mass of the seedlings, the toxicity changes in the following order: p-nitroaniline < Control < 2,4-DNP < picric acid < 2,6-DNP < 2,5-DNP < m-nitrophenol. It can be noticed from the table 1 that m-nitrophenols affect the most the mass of the seedlings, 17.54 mg in comparison with 44.8 mg - the mass of the control seedlings. An unusual result for these experiments was that the mass of the seedlings intoxicated with *p*-nitroanaline is approximately twice the mass of the control

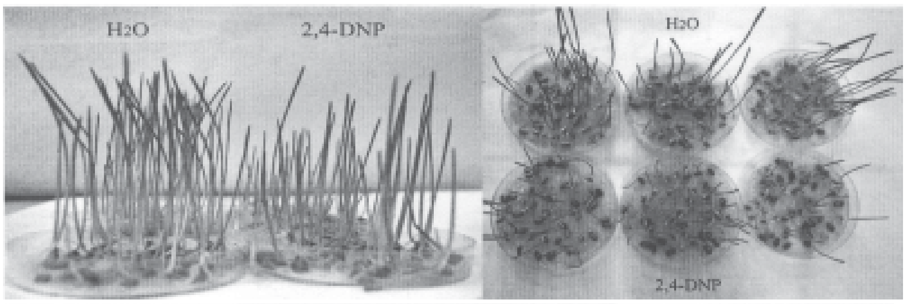

Fig. 3. The effect of 2,4-DNP on the wheat seeds' germination vs. control samples

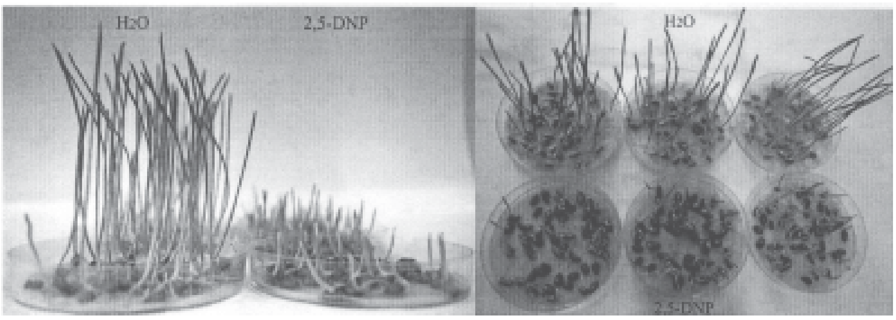

Fig. 4. The effect of 2,5-DNP on the germination of wheat seeds

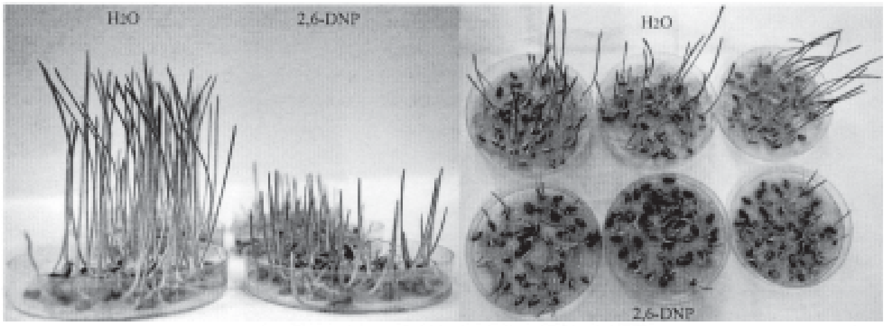

Fig. 5. The effect of 2,6-DNP on the germination of wheat seeds

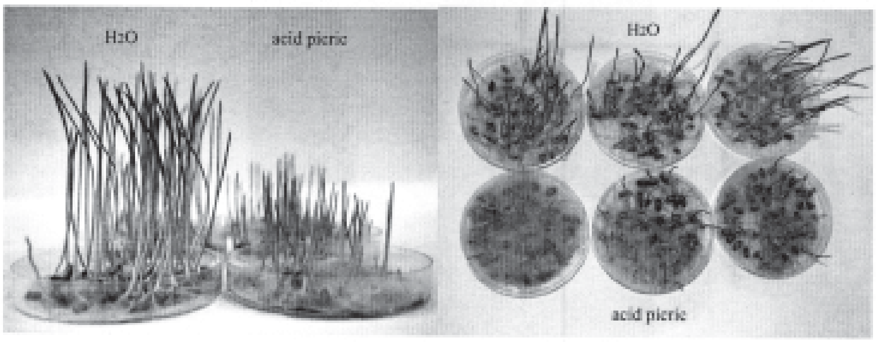

Fig. 6. The effect of picric acid on the germination of wheat seeds

seedlings, although the over studied parameters suggest that p-nitroanaline is toxic for the wheat seeds.

In order to point out the changes that take place during 7 days of germination, at the level of the development rate of the wheat seeds, a comparative study was realised: nitroderivative (samples treated with nitroderivatives) vs. standard (control samples with distilled water).

From figure 3 can be noticed that 2,4-DNP inhibits the growth rate of the seedlings, and in this case of wheat seeds treated with 2,4-dinitrophenol a 37.2% of dead seeds was obtained, while the standard presented only 27.2% of dead seeds. Also, in comparison with the standard, the wheat seeds treated with 2,4-dinitrophenol presented an average height smaller with 1.96 cm, and a decrease in mass with 7.17 mg.

The toxicity of 2,5-dinitrophenol can be observed especially in the height of the seedlings, the seedlings presented an average height of 1.28 cm, which is smaller with 5.72 cm than the control seedlings. A significant difference can be noticed also for the unitary mass which is with 24.31 mg smaller and the percent of the dead seeds is of 38.6% in comparison with 27.2 % of the control seeds (fig. 4).

In case of wheat seeds treated with 2,6-DNP, 44% of seeds were found dead, the average unit mass was 23.32 mg and the seedlings presented an average height of 2.66 cm. The differences between the seeds treated with 2,6-DNP and the seeds treated with double distilled water (standard) are considerable (fig. 5), the last one having an average height 3 times bigger and the unit mass is double in comparison with seeds intoxicated with 2,6-DNP.

The picric acid inhibited the seedlings growth and only 51.2 % of the seeds germinated. The seedlings presented an average height of 2.92 cm and an average unit mass of 29.30 mg. In case of seeds treated with picric acid, the appearance of mould was observed. This phenomenon wasn't observed when the seeds were treated with other investigated nitrophenols (fig. 6). It is possible that the nutrients were absorbed mostly by the mould (beneficial nutritive environment for mould growth).

When intoxicated with m-nitrophenol (fig. 7), the resulted seedlings presented a 56.6% percentage of growth, an average height of 2.73 cm and an average unit mass of 17.54 mg. To be noticed that the seeds treated with m-nitrophenol had the smallest unit mass among the seeds intoxicated with other aromatic derivatives and with the

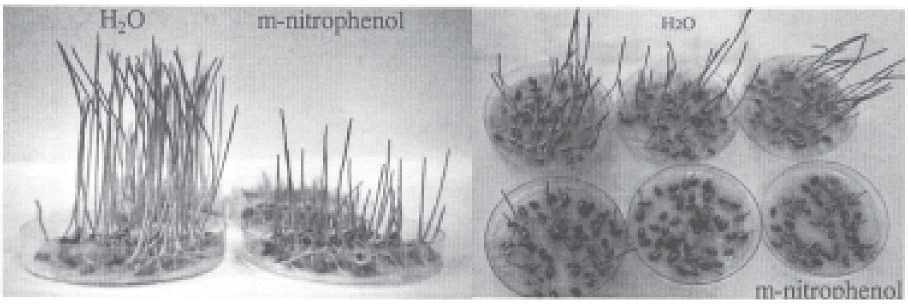

Fig. 7. The effect m-nitrophenol on the germination of wheat seeds

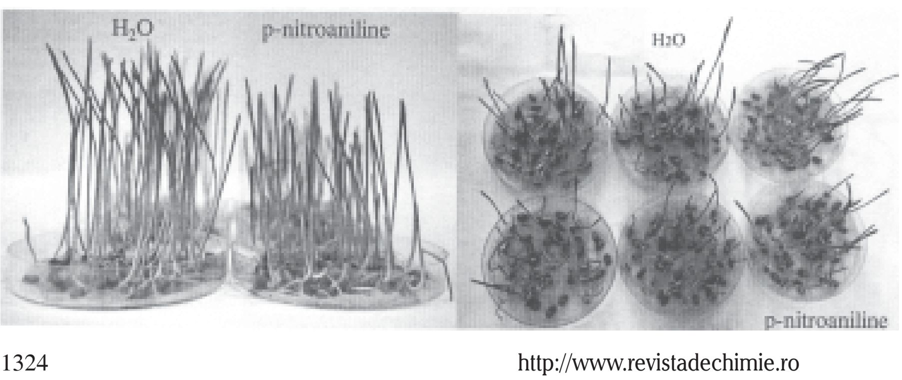

Fig. 8. The effect of *p*-nitroaniline on the germination of wheat seeds

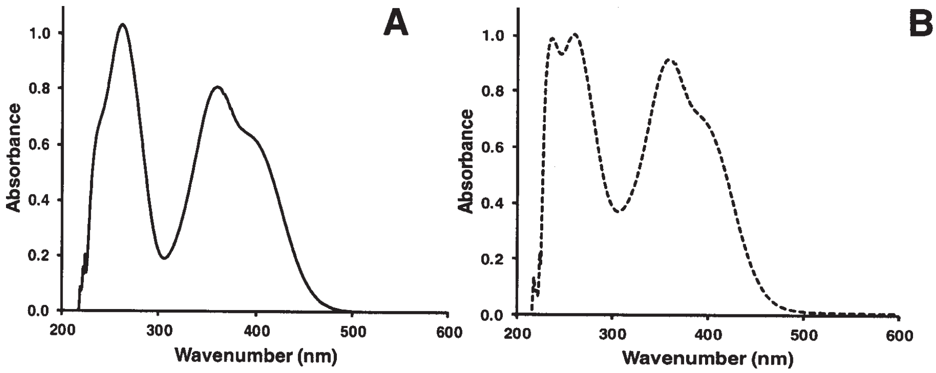

Fig. 9. Absorption spectra of: **A)** 2,4-DNP ($5x10^{-4}$ M) and **B)** 2,4-DNP (supernatant) 5.65 $x10^{-4}$M

average unit mass id with 26.54 mg smaller than the average unit mass of the control seeds.

In figure 8 is presented the effect of *p*-nitroaniline on the wheat seeds. From the conducted experiment can be observed that p-nitroaniline is the chemical compound that inhibited least the growth of the wheat seeds. The seedlings presented a growth percentage of 61.2%, an average height of 5.1 cm and an average unit mass of 82.19 mg. This unit mass value is approximately twice the value of average unit mass corresponding to control seeds treated with double distilled water.

UV-Vis absorption studies

The pure 2,4-dinitrophenol presented 2 absorption maxima at 262 nm (A = 1.033) and 360 nm (A = 0.807) (fig. 9A). As well, 2 shoulders at 240 nm (A = 0,695) and 378 nm (A = 0.694) can be observed. In UV-Vis spectrum of aqueous 2,4-DNP solution resulted after seeds treatment can be observed 3 absorption maxima at 224 nm (A = 0.222), 237 nm (A = 0,988) and 359 nm (A = 0.913) and a shoulder at 380 nm (A = 0.765) (fig. 9B).

The difference between the 2 spectra is obvious, in case of the solution resulted after the treatment, a third absorption maximum arises and it could be due to the interference determined by the wheat seeds. Also, it was observed that the concentration of 2,4-DNP in this two solutions is different, the resulted treatment solution appeared to be more concentrated.

The absorption of the treatment solution by the wheat seeds

In order to find an explanation for the fact that the concentration of 2,4-DNP seems to increase after treating the seeds, gravimetric measurements on wheat seeds were performed. The wheat seeds had an initial mass m_i = 2.363 g, after the treatment the seeds' mass became of 3.624 g. After drying the seeds on filter paper, a finale mass m_f = 3.276 g was obtained. The wheat seeds absorbed 0.913 g of treatment solution. This means that the seeds absorbed 0.913 mL from 5 mL of the treatment solution if assuming that the density of the treatment solution is approximately 1 g/cm^3.

Conclusions

In this paper was demonstrated the toxicity of dintrophenol pesticides for the viability of the plant material. These experiments revealed the sensitivity of the wheat seeds towards dinitrophenols that inhibited the seeds growth. The effect of the treatment with aromatic derivative is better reflected by the individual seedlings heights and by the average unit seeds mass. The toxicity effect measured as number of non-germinated seeds is not considered and in this case are studied only the individual plants that suffered from the treatment. Each compound affects specifically the plant's development, and it is known that every living organism reacts in a specific manner to external stimuli. Thus, the dead seeds were not considered when determining the individual height and the average unit mass.

In other experiment were compared the spectra corresponding to the initial 2,4-dinitophenol solution, and the solution resulted after the treatment of the seeds; differences were observed between the spectra suggesting that the plants absorbed from the 2,4-DNP solution. It was also noticed that plants have the tendency to protect themselves, absorbing more water than toxic from the solution. Nowadays, the interest in research for dinitrophenols is great because it can have a great impact on treatment of some diseases; still these compounds are considered a poison without an antidote, and the toxicity mechanism hasn't been completely elaborated.

This type of studies is extremely important for the genetic conservation of endangered species, which are conserved in gene banks and reservations. The conservation of genetic resources in gene banks is considered the most advanced form. The Gene Banks are specialized institutions responsible for the collection, evaluation and conservation of genetic resources. The Gene Bank has the role to conserve plant germplasm from Romania and thus to contribute to the assurance of global plant genetic resources. Plant genetic resources contribute to food safety, being the most valuable gift gives us from nature.

Acknowledgements: Financial suport within project from the Romanian Government (PN-II-PT-PCCA-2013-4-1149, Contract 107/ 2014). Andrieş Claudia's work was supported by the strategic grant POSDRU/159/1.5/S/133652, co-financed by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007-2013.

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Manuscript received: 17.02.2015