# THE INFLUENCE OF THE ULTRASOUND TREATMENT ON SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN *SPINACIA OLERACEA* L. SEEDS

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Keywords: Spinacia oleracea, ultrasounds, respiration intensity, catalase

**Abstract:** The paper presents the results of a study regarding the respiration intensity and the activity of the catalase in the *Spinacia oleracea L*. seeds, subject to the action of ultrasounds with the frequency of 23 and 36 kHz, the electrical power of 7,4; 49,7; and 30 V.A., at the time intervals 20, 30, 40 and 60 seconds. The results obtained emphasize a stimulation effect of the germination of seeds and a slight intensification of the activity of catalase and the respiration intensity.

#### **INTRODUCTION**

The ultrasounds are acoustic waves with higher frequencies than the audibility threshold of 16000 Hz (Dimitriu et al., 1990). Because of the high frequencies and the high energy that they release in the propagation media, the ultrasounds produce a series of physical-chemical effects which lay at the basis of their use in different fields: physics, chemistry, biology, medicine, agriculture, industry etc.

The researches carried out by a series of authors (Ausländer et al., 1966; Albu et al.1968; Raianu and Zanvetor, 1969; Dăbală et al., 1970; Albu, 1980) have emphasized the stimulating effect of ultrasounds on the germination, growth and development of plants and also on the vegetal production. In addition, the experiments carried out by us with the seeds of *Pastinaca sativa* have emphasized a stimulating effect of ultrasounds on the intensity of respiration and activity of some oxidoreductases (Stratu et al., 2005). We determined the fact that the sensitivity of seeds to the treatment with ultrasounds varies according to species, used acoustic parameters, time of exposal and morphophysiological characteristics of the seeds.

Based on these considerations, we proposed to test the effect of the ultrasound treatment on *Spinacia oleracea* seeds – species which germinates with more difficulty and has a lower germination faculty.

### **MATERIAL AND METHOD**

As biological material we used *Spinacia oleracea* seeds from 2007 harvest, acquired from Unisem Iași. The seeds, introduced in the water, were subjected for ultrasound field treatments by two modalities:

- by using an electric high frequency generator with the possibility to modifying in stages the electric power;

- by using an ultrasound bath.

We achieved ten experimental variants (four control variants and six treatment variants) according to the values of the used acoustic parameters and exposal time (Table 1). After carrying out the treatment, the seeds were placed in Petri plates for germination under laboratory conditions.

Table 1. The experimental variants				
Crt. no.	Experimental	Exposal time	Acoustic parameters	
	variants	[s]	Frequency	Electric power
			[kHz]	[W]
1.	M1	20	-	-
2.	M2	30	-	-
3.	M3	40	-	-
4.	M4	60	-	-
5.	V1	20	23	49.7
6.	V2	30	23	49.7
7.	V3	40	23	7.4
8.	V4	60	23	7.4
9.	V5	40	36	30
10.	V6	60	36	30

Table 1. The experimental variants

In these experiments we have studied the seeds respiration intensity and catalase activity.

The seeds <u>catalase</u> activity was determined by iodometric method which consists, in principle, in the evaluation of the peroxide consumption in a determined time interval (Artenie and Tanase, 1981).

The seeds respiration intensity was evaluated by Warburg method (Boldor et al.1981)

The investigated parameters were determined at 6, 8, 10, 12 and 14 days since the beginning of the experiment.

## **RESULTS AND DISCUSSIONS**

Spinach (*Spinacia oleracea*) is an herbaceous, annual plant in the family of *Chenopodiaceae*, with a large nutritional (rich source of iron, calcium, vitamin A, vitamin C, vitamin E, vitamin K, vitamin B<sub>9</sub>, niacin, opioid peptides, omega 3-fatty acids, magnesium, selenium and several vital antioxidants) and medicinal (remineralized, antianemic, antiscorbutic) values, and melliferous, originating from Central and southwestern Asia, cultivated since the IV century. The fruit is a spherical, edgy or thorny achene, of greenish, grayish or fawn color.

The reserve substances of the spinach seeds are represented by 24% proteins and 7% lipids (Burzo et al., 2000).

The observations made during the deployment of experiments emphasized an effect of stimulating the germination of seeds in the case of variants treated with ultrasounds.

The seeds respiration intensity during the germination process reflects the physiological activity of the embryo and represents an indicator of the metabolic activity and the stress state.

In all the experimental variants, the respiration intensity has, generally, a similar dynamics: it present high values at 6, 8, 14 days and reaches the maximum value in the  $12^{th}$  day since the beginning of the experiment (figures 1, 2 and 3).

Comparatively to the control, in the variants treated with ultrasounds we notice values similar to it, in some cases, slightly higher.





Figure 1. The respiration intensity in *Spinacia* oleracea ultrasounds treated seeds – V1 and V2 variants

Figure 2. The respiration intensity in *Spinacia* oleracea ultrasounds treated seeds – V3 and V4 variants



ultrasounds treated seeds – V5 and V6

The tendency of stimulating the respiration intensity is more evident in the following variants, presented in the decreasing sequence: V4, V3 (the electric power: 49,7 V.A.; the

frequency:23 kHz; the time of exposal: 40s and 60s) ; V6, V5 (the electric power :30 V.A.; the frequency: 36 kHz; the time of exposal: 40s and 60 s.).

The favorable influence on the respiration is due to the mechanical effects that contribute to the tegument corrosion and the increase of the water absorption capacity and chemical effects that accelerate the oxidoreduction processes.

The control respiration dynamics confirms the data from the specialty literature mentioned for the analyzed species and for other species such as: *Lactuca sativa, Zea mays, Glicine max, Lathyrus odoratus.* (Bewley and BlacK, 1978, cited by Burzo et. al., 1999).

The catalase is an enzyme from the group of oxidoreductases. Through the peroxidase action (supplementary to the catalytic function), the catalase intervenes in the respiration of plants, being indirectly an indicator of the metabolic activity.

In the analyzed interval the catalase activity has a similar activity (with increasing and decreasing stages) in all the experimental variants. The most intense enzymatic activity was determined in the 12<sup>th</sup> day since the beginning of the experiment (figures 4, 5 and 6).



Figure 4. The catalase activity in *Spinacia oleracea* ultrasounds treated seeds - V1 and V2 variants



Figure 5. The catalase activity in *Spinacia oleracea* ultrasounds treated seeds - V3 and V4 variants



Figure 6. The catalase activity in *Spinacia oleracea* ultrasounds treated seeds – V5 and V6 variants

The oscillations in the catalase activity are in connection with the degradation processes of the endosperm and cotyledons reserve substances and synthesis of some necessary substances for nutrition and embryo growth. On the other hand, they could be determined by the treatment with ultrasounds which intensify the enzymatic activity.

In comparison with the control, we determine a stimulation of the catalase activity in the V4 variants (at 6, 8, 10 days since the experiment beginning); V2 (at 8, 10, 12 days since the

experiment beginning); V5 (at 6 and 10 days since the experiment beginning) and V6 (at 6 and 12 days since the experiment beginning).

The results obtained in the case of the control variants confirm the data from the literature, according to which, the catalase activity increases a lot since the germination beginning and reaches a maximum after a certain number of days, according to the species, after which it begins to decrease (Burzo et. al., 2000).

#### CONCLUSIONS

Our results emphasize specific value variants of the catalase activity and respiration intensity according to the ultrasound parameters used and the exposal time.

The seeds tested "respond" to the treatment with ultrasounds through a slight intensification of the catalase activity and the respiration process.

The effects of the treatments with ultrasounds noticed in the case of experiments carried out with the test species *Spinacia oleracea* confirm the data from the specialty literature determined for other species with economic value.

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