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Study of the influence of some physical factors on the viability of seeds of *Echinaceae pallida* variety "Lebedushka"

Application of physical factors allows activating seed germination and increasing plant yield. The article presents data on testing of germination parameters of seeds of *Echinaceae pallida* variety "Lebedushka" under the influence of physical factors. Seeds were preliminarily immersed in liquid nitrogen for a day, and then subjected to laser irradiation, babbling and magnetic fields for a certain time. As a result of the experiments, it was determined that exposure to a constant magnetic field promoted a reliable increase in germination rates by 10.4 % compared to the control. The best germination was determined in seeds exposed to a constant magnetic field with an induction of 75 μTesla for 3 days, without prior cryopreservation (90.0 %). In the experiments with bubbling, the best germination was determined in seeds without cryopreservation – 75.25 %, and in the variant of the experiment with He-Ne laser irradiation for 2 minutes without preliminary immersion in liquid nitrogen vapor – 95.0 %.

Keywords: seeds, *Echinacea pallida*, magnetic fields, babbling, radiation by He-Ne laser, cryogenic storage, germination.

Introduction

Consideration of the influence of magnetic fields and laser irradiation on the preservation of viability of seed material of medicinal plant species is currently of great theoretical and applied importance. Valuable medicinal plants provide pharmaceutical plants of Kazakhstan with medicinal plant raw materials.

According to some literature data [1], a positive effect of various physical factors on the viability of germinating seeds and the passage of seedlings through the phases of ontogenesis of economically useful plants has been established.

Laser irradiation acts as a bio stimulator [2], as it triggers mechanisms of growth and development of plant organism, but this physical factor is poorly studied in relation to herbs. In the scientific environment there are data of laboratory studies proving the postive effect on the germination of seed material. With laser bio stimulation of seeds, an increase in the yield of various crops is observed. Due to ionizing radiation there is an energy exchange between endosperm and embryo, and absorption of rays occurs by certain structural components of the seed coat.

It is advisable to use the bubbling method for seeds with germination of less than 70 % [1]. Germination and germinative energy increase as a result of better permeability of the protective layer of the seed coat, as well as washing out of various pathogenic floras by air bubbles. Due to bubbling, seeds come out of deep dormancy faster; as a result they germinate 1-2 days earlier than in control variants. Seed material is placed in a vessel with water for one day, where as a result of compressor operation, oxygen bubbles are formed. Advantageous features of this method are the rapid passage of ontogenesis phases, friendly germination of seeds, and high biological productivity of plants.

Under the influence of magnetic field [3], the biological clock of plants, the main processes of synthesis of various organic compounds are started. The publications of research scientists contain data on the positive effect of magnetic fields on the viability of seed material of various plant species.

Currently, the number of most plant species, including medicinal plants, growing in Kazakhstan is decreasing due to the impact of anthropogenic factor [4, 5].

There is a need to preserve the gene pool of plants, which is based on the data of scientific research conducted in various countries, and one of the promising methods is cryogenic storage of plant germplasm in liquid nitrogen [6, 7].

To date, there is a small number of scientific works devoted to the study of the influence of various physical factors on the viability of seeds and biology of germination of seedlings of medicinal plants. The main source of medicinal plant raw materials for the pharmaceutical industry is medicinal plants growing in natural habitats or cultivating species. One of the promising medicinal plants is *Echinacea pallida*, which has antioxidant, antiradical, anti-mutogenic, radioprotective and immunostimulating properties, antimicrobial activity due to the presence of water-soluble polysaccharide complex relative to *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Candida albicans* [5, 8].

Experimental

Seed material of *Echinacea pallida* variety "Lebedushka" was used in the experiments. Laboratory experiments were conducted to study the effect of physical factors on germination and germination biology of seeds of *Echinacea pallida* variety Swan.

To study the effect of babbling, seeds were packed in cloth bags and immersed in a plastic cylinder filled 2/3 with water, compressed air was pumped by an aquarium compressor. Several variants of the experiment were laid down: 1) with preliminary cryogenic storage for a day in a Dewar vessel and subsequent bubbling process for 24 hours; 2) seed bubbling for a day.

In the magnetic field experiments, seeds were packed in unsealed plastic tubes that were placed in a magnet setup. A constant magnetic field of 75 µTesla or 150 µTesla induction was continuously applied to the test seeds in the magnetic setup. The direction of the magnetic field of the setup coincided with the direction of the Earth's magnetic field. Two variations of experiments were applied: with pre-storage for a day in liquid nitrogen vapor and without cryopreservation. Seeds were irradiated for a day and 3 days.

Irradiation with He-Ne laser with a wavelength of 632.8 nm and intensity of 5mW/cm^2 was carried out for a certain time: 30 seconds, 1, 2 and 4 minutes. Experiments with cryogenic storage and subsequent exposure to laser irradiation and without cryopreservation were also conducted with the seeds.

Seeds were frozen in cryobiological tubes at -196 °C for a day in Dewar vessels, followed by slow thawing at room temperature [9].

Seeds of the studied species were sown immediately after the manipulations. Seeds were germinated in Petri dishes on 2 layers of filter paper moistened with water in 4-fold repetition. Seeds were disinfected with 0.5 % potassium permanganate for 5-6 minutes. Petri dishes with planted seeds were placed at a temperature of +24°C in a climatic chamber.

Seed germination parameters were studied according to the methodological instructions of M.S. Zorina and S.P. Kabanov [10], M.V. Maltseva [11]. The development of seedlings was monitored for 14 days. Statistical processing of the obtained data was performed according to the method of N.L. Udolskaia [12] and using the site medstatistic.ru [13].

Results and Discussion

The comparative analysis of the effect of permanent magnetic field on the viability of seeds of *Echinacea pallida* variety "Lebedushka" showed that the best germination was demonstrated by seeds without preliminary cryopreservation and subjected to continuous exposure to a permanent magnetic field with an induction of 75µTesla for 3 days – 90.0 %; similar germination was shown by seeds with preliminary cryopreservation for a day and subsequent exposure to a permanent magnetic field with an induction of 150µTesla for 3 days. The control group showed germination of 75.0±5.77 %. The best indicators of germination without preliminary cryopreservation were observed in seeds exposed to a constant magnetic field induction of 75µTesla for 3 days – 90.0 %. Compared to the control group, the average germination increased by 11.7 % with cryopreservation, and by 8.2 % without cryopreservation. In the experiment with preliminary cryogenic storage for a day and subsequent exposure to a magnetic field, the best germination was observed in seeds exposed to a constant magnetic field with an induction of 150 µ Tesla for 3 days – 90.0 % (Table 1).

Table 1

Parameters of germination of *Echinacea pallida* variety "Lebedushka" seed materials in different experiments

Parameters of germination, %	Control	Without cryopreservation				After cryopreservation			
		Magnet field 75 µTesla		Magnet field 150 µTesla		Magnet field 75 µTesla		Magnet field 150 µTesla	
		Period of treatment		Period of treatment		Period of treatment		Period of treatment	
		1 day	3 days	1 day	3 days	1 day	3 days	1 day	3 days
Germinative energy, %	65.0± 6.5	75.5± 3.21	86.25± 2.76*	60.5± 6.35	54.25± 2.51	78.0± 3.13	65.5± 9.68	75.0±10	75.0± 3.33
Seed germination, %	75.0± 5.77	85.25± 3.18	90.0*	85.0± 3.33	75.0± 3.33	80.5± 0.58	77.5± 8.66	85.0± 3.33	90.0*

**reliable difference between the results of the experiment and the control at P≤0.05*

As a result of the analysis of the obtained data of laboratory studies, the positive effect of exposure to a constant magnetic field with induction from 75 µTesla to 150 µTesla on the viability of seed material and the passage of ontogenesis phases was determined. Germination improved on 15 % compared to the control data.

Thus, having considered the effect of a constant magnetic field with induction from 75 µTesla to 150 µTesla on the germination parameters of seeds of *Echinacea pallida* variety "Lebedushka", it can be noted that the viability of seed material improved compared to control values by 9.75 %. In all variants of the experiment the seedling passes all phases of ontogenesis, in some variants of the experiment there was a delay in germination of seed material for 1-2 days.

For long-term storage of seeds it is recommended to apply preliminary cryogenic storage for a day with subsequent activation in a permanent magnetic field with an induction of 150 µTesla for 3 days.

At the next stage, a series of experiments on babbling of seed material was carried out. The germination comparable to control values was established. In the experiment with pre-cryopreservation and subsequent babbling, the germination decreased to 65.0±3.33 % compared to the control. In the experiments with babbling, germination was 75.25±3.51 %, which is 0.25 % higher than the values in the control group (Table 2)

Table 2

Parameters of viability of *Echinacea pallida* variety "Lebedushka" seeds after babbling treatment

Variant of experience	Germinative energy, %	Seed germination, %
Control	65.0±6.5	75.0±5.77
Babbling	60.0±6.34	75.25±3.51
Cryopreservation + babbling	60.0±6.67	65.0±3.33

Analyzing the dynamics of seed germination it was found that seeds germinate faster on the day in the experiment variants with barbotage – on the 2nd day, in the control group on the 3rd day (Fig. 1).

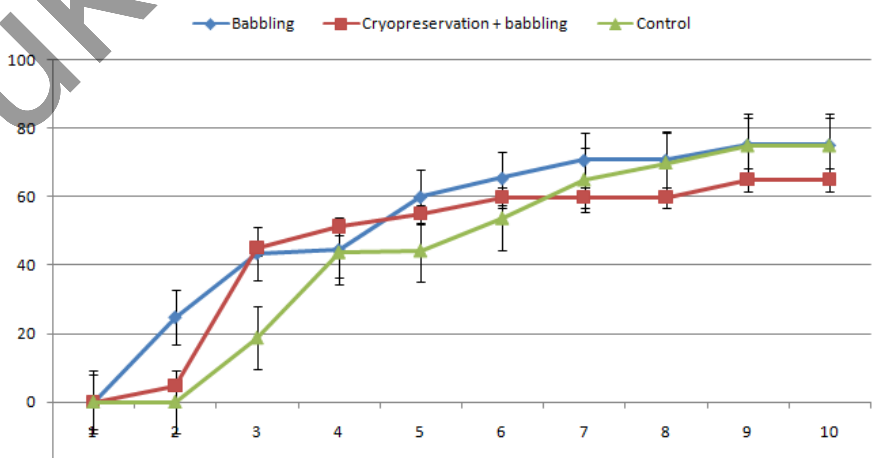

Figure 1. Dynamics of germination of seed material of *Echinacea pallida* variety "Lebedushka" after babbling treatment

The effects of laser radiation on the biology and dynamics of seed germination were examined in laboratory experiments. Seed material of *Echinacea pallida* was exposed to He-Ne laser for a duration of 30 seconds to 4 minutes. The best germination was demonstrated by seeds without preliminary cryogenic storage, irradiated for 2 minutes – 95.0±3.33 %, which is 20 % higher than the control values (Table 3).

Table 3

Influence of laser radiation on seed viability of *Echinacea pallida* variety "Lebedushka"

Parameters of germination	Control	Without cryopreservation				With cryopreservation			
		Period of treatment		Period of treatment		Period of treatment		Period of treatment	
		30 sec	1 min	2 min	4 min	30 sec	1 min	2 min	4 min
Germinative energy, %	65.0± 6.5	60.0± 6.67	64.5± 16.34	85.0± 3.33	60.0±0	55.0± 7.01	90.0± 6.67*	50.0± 6.67	40.0±0*
Seed germination, %	75.0± 5.77	70.0± 6.67	70.5± 1238	95.0± 3.33*	85.0± 3.33	66.75± 6.84	90.0± 6.67	60.0±0*	50.0± 6.67
** reliable difference between the results of the experiment and the control at P≤0.05*									

By conducting a comparative analysis of the conducted studies with and without preliminary storage of seeds for a day in liquid nitrogen vapor, followed by exposure to laser radiation, it was found that the best viability was demonstrated by seeds without cryopreservation. Thus, in experiments with seeds without cryopreservation, the best germination rates were observed in seeds irradiated with laser beam for 2 minutes – 95.0±3.33 %. In experiments with pre-cryopreservation, the best germination was determined in seeds irradiated for 1 minute – 90.0±6.67 %.

Having studied the dynamics of germination of seeds of *Echinacea pallida* variety "Lebedushka" in different variations of the experiment, it was determined that seeds irradiated for 30 seconds without preliminary cryopreservation begin to germinate on the 3rd day from the moment of sowing, on the 4th day – exposed to laser radiation for 2 and 4 minutes without cryopreservation and with preliminary cryogenic storage and subsequent irradiation of 30 seconds. In the remaining experiments, germination was observed on the 5th day.

The effect of physical factors on germination parameters of *Echinacea pallida* variety "Lebedushka" was considered and analyzed, it was found that to obtain viable seed material and well-developed seedlings should be exposed to laser beam for 2 minutes, germination in this variant of the experiment increases by 20 % compared to the control (Table 4).

Table 4

Effect of different physical factors on seed viability parameters of *Echinacea pallida* variety "Lebedushka"

Parameters of germination	Control	Permanent magnetic field B=75mTesla (3 days) without cryopreservation	Babbling	Without cryopreservation, He-Ne laser irradiation for 2 minutes
Germinative energy, %	65.0±6.6	86.25±2.76*	60.0±6.34	85.0±3.33*
Seed germination, %	75.0±5.77	90.0±0*	75.25±3.51	95.0±3.33*
** reliable difference between the results of the experiment and the control at P≤0.05*				

Comparing the effect of physical factors on the dynamics of germination it was found that seeds begin to germinate on the 2nd day using the method of babbling, on the 4th day – with laser beam irradiation for 2 minutes, on the 5th day – with exposure to a constant magnetic field with an induction of 75 µTesla for 3 days (Fig. 2).

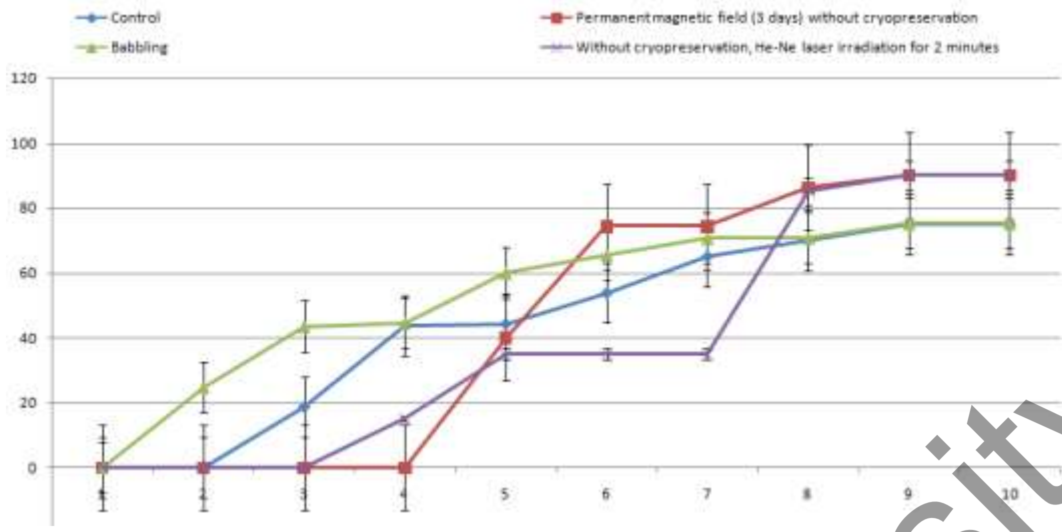

Figure 2. Effects of physical factors on the dynamics of seed germination of the test species *Echinacea pallida* variety "Lebedushka"

Conclusion

According to the analyzed literature review, it was determined that physical factors have a positive effect on the viability of seeds of the studied species. Permanent magnetic field with induction from 75 µTesla and up to 150 µTesla has a favorable effect on germination indices of seeds of the studied species: germination increased by 10.4 % compared to the control. The best germination was observed in seeds exposed to a permanent magnetic field of 75 µTesla induction for 3 days, without preliminary cryogenic storage – 90.0 %. In experiments with bubbling, the best germination was found in the variant without cryogenic storage – 75.25±3.51 %. In the experiment with laser irradiation, the best results were obtained in the variant of irradiation for 2 minutes without preliminary cryopreservation – 95.0±3.33 %. Thus, the use of He-Ne laser for 2 minutes to activate germination can be recommended for the seed material of *Echinacea pallida* variety "Lebedushka".

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Echinaceae pallida сортының «Лебедушка» тұқымдарының өміршеңдігіне физикалық факторлардың әсерін зерттеу

Мақалада физикалық факторларға ұшыраған *Echinaceae pallida* сортының «Лебедушка» тұқымдарының өнуі туралы ақпарат берілген. Тұқымдар алдын-ала бір тәулік бойы сұйытылған азотқа батырылды, содан кейін белгілі бір уақыт бойы лазерлік сәулеленуге және магнит өрісіне ұшырады. Сонымен қатар зертханалық зерттеулерде көпіршік әдісі қолданылды. Тәжірибелердің нәтижесінде магнит өрісінің әсері бақылаумен салыстырғанда өну жылдамдығының 10,4 %-ға артуына ықпал ететіні анықталды. Ең жақсы өнгіштік алдын ала криосақтаусыз 3 күн бойы бір магнит өрісінің әсеріне ұшыраған тұқымдарда табылды, ол — 90,0 %. Көпіршіктенумен жүргізілген тәжірибелерде ең жақсы өну криоконсервациясыз тұқымдарда — 75,25 %, ал He-Ne лазерімен сәулелендіру тәжірибесінде алдын-ала сұйық азотқа батырылмағандықтан 2 минут ішінде — 95,0 % болды.

Кілт сөздер: тұқымдар, *Echinaceae pallida*, магнит өрісі, барбортирлеу, He-Ne лазермен сәулелену, криогенді сақтау, өнімділік.

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Изучение влияния физических факторов на сохранение жизнеспособности семян *Echinaceae pallida* сорта «Лебедушка»

В статье представлены сведения о прорастании семян *Echinaceae pallida* сорта «Лебедушка», подвергшихся воздействию физических факторов. Семена предварительно погружались в жидкий азот на сутки, затем подвергались лазерному облучению и воздействию магнитных полей в течение определенного времени. Также в лабораторных исследованиях был использован метод барботирования. В результате проведенных экспериментов было определено, что воздействие постоянного магнитного поля способствует улучшению показателей прорастания на 10,4 % по сравнению с контролем. Наилучшая всхожесть была определена у семян, подвергшихся воздействию постоянного магнитного поля индукцией 75 мкТесла в течение 3 суток, без предварительного криохранения, — 90,0 %. В экспериментах с барботированием лучшая всхожесть была установлена у семян без криоконсервации — 75,25 %, а в варианте эксперимента с облучением He–Ne лазером в течение 2 мин без предварительного погружения в пары жидкого азота — 95,0 %.

Ключевые слова: семена, *Echinacea pallida*, магнитное поле, барботирование, облучение He–Ne лазером, криогенное хранение, всхожесть.

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