Effect mechanisms of ultrahigh-frequency radiation on biological objects

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Abstract. The article discusses the effect of microwave radiation on the seeds of Scots pine (Pinus sylvestris L.). The aim of the research is to increase the efficiency of pre-sowing treatment of seeds with Ultra-high-frequency radiation (UHF) radiation, allowing one to increase the standard planting material yield and reduce its cultivation time. The specificity of enzyme systems for stressing effects is revealed, the dynamics of their change in the period after irradiation is ambiguous and depends on the time of irradiation. The stimulating and lethal doses of radiation are determined. The results show seed treatment with stimulating doses causes reversible changes in the structure and function of enzymes. The regularities and model of the impact of UHF radiation as a stimulating factor on seed viability are established. When exposed to stress factors (radiation), an increase in the activity of enzymes is observed, leading to the excitation or inhibition of growth processes at the first stages of development. As a result, a stimulating effect arises – the germination of seeds increases, the growth of seedlings in height increases, or depressing – the germination decreases and the growth of seedlings slows down.

1. Introduction

The most effective and common way to create artificial plantations is planting. Currently, the specific gravity of this method is about 80%. Artificial afforestation and urban greening planting material is grown in nurseries. In relation to the increase in the volume of forest planting work, the cultivation of planting material in forest nurseries is of exceptional importance, the quality of which largely determines the efficiency of work on the artificial reproduction of forest resources. Standard planting material production requires seeds.

High physiological activity characterizes tree and shrub species seeds under development. Their tissues contain a large amount of mobile carbohydrates and nitrogen compounds, which, as the seed matures, accumulate in it in the form of starch, protein, and fats. When the seed is fully matured, its physiological activity slows down, the seed embryo stops growing, the movement of nutrients stops, and the water content decreases. Creating conditions under which the physiological activity of seed tissues and the growth of the embryo will resume is necessary for the germination of the seed.

For economic reasons, in most cases, seeds are not sown after collection, they are stored for a certain time. At this particular time, the natural order of seed germination preparation is disrupted, as a result of which the emergence of seedlings is delayed, the soil germination of seed and the quality of plants grown from seeds is reduced. To avoid this, in forestry, seeds are treated before sowing, i.e. pre-sowing treatment of seeds is done.
The most common methods of pre-sowing seed treatment are stratification, seed soaking, seed treatment with microelements, hydrothermal action, scarification, disinfection and disinsection. However, these methods mainly serve to overcome the dormant period of the seed and do not provide high survival rate and rapid growth of seedlings. The use of Ultra-high-frequency radiation (UHF) radiation of seeds of Pinus sylvestris L. is a more progressive pre-sowing seed treatment way, allowing to remove seeds from dormancy and to activate the work of various biological catalysts - enzymes that ensure rapid growth and development of seedlings. Among the entire spectrum of electromagnetic radiation (EMR) of the radio wave range, ultrahigh-frequency EMR or microwaves have a pronounced biological effect, which includes the entire wave range that occupies an intermediate position between ultrahigh-frequency and optical. At the same time, the "thermal effect" of microwaves associated with an increase in the temperature of biological tissue is most well studied [1-3]. In biological objects, microwaves also cause a non-thermal effect, when resonant effects are formed when cells are irradiated in low-frequency fields, against the background of a general increase in temperature [4-7].

The physical, mechanical, and biological mechanisms of EMR influence are not yet fully understood. First, the energy of the microwave EMR quantum, even in the short-wave millimeter range, does not exceed 10 eV, which practically eliminates significant quantum effects at the level of microparticles. Secondly, the theoretical and experimental foundations of this problem were first developed by biologists, not physicists. In a biological object, resonant absorption of microwave radiation energy is theoretically possible in the entire wave range: decimeter, centimeter, and millimeter [8-11].

The scientific justification of the possibility of using the EMR of the millimeter range of low power on living organisms of different complexity of the organization was given by N. D. Devyatkov [12]. The results of the EMR action depend on the choice of the biological object, on its initial state, on the irradiation conditions (primarily on the wavelength). Moreover, all the observed effects can be divided into two large groups. The first groups is characterized by an acutely resonant nature of the response of organisms to radiation, the second is characterized by the fact that the biological effect of exposure appears at a certain minimum intensity of radiation and does not change when the intensity of radiation changes over a wide range.

The paper is focused on the reaction of Pinus sylvestris L. seeds to the effects of microwave radiation at the cellular and subcellular levels. The aim of the work is to increase the germination and energy of seed germination and growth as a result of pre-sowing seed treatment with microwave irradiation.

2. Materials and methods
According to the goal of the research, it was necessary to plan the experiment in such a way as to best predict the effective doses and measure the values of exposure to microwave radiation that affect the germination and energy of seed growth. The quality of the seed material was determined according to ISTA [13, 14]. To study the effect of microwave radiation on seed germination, seeds of Pinus sylvestris L. collected from a royal family plantation of Voronezh origin were used. The cones were harvested in April. The opening of the cones was maintained at room temperature (22-25°C) during April-May. The purity of the seeds was 99%, the moisture content of the seeds was 70%. Experiments on processing and germination of seeds were conducted in May-June.

In the experiment on microwave irradiation of seeds and seedlings, a microwave drying oven with a frequency of 2.45 GHz and a generator power of 35 kW was used. The volume of the furnace is 3 m³, which allows you to irradiate the seeds in large batches. Exposure time from 1 to 13 min. Seeds, germinated without preliminary soaking, were washed with tap water at 18-20°C on a metal mesh for 10-15 sec immediately before analysis. A machine for germinating seeds in the light with an automatic regulator to maintain a constant temperature and Petri dishes were used. The machine consists of a metal case of any size, filled with water, inside which an electric heating coil runs; top housing covered with metal trays, which are placed on the bed for sprouting seeds. The bottom of the device, inner walls, sheets and trays were washed with water and doused with boiled water at a temperature of
more than 95°C. As a bed, we used linings with wicks made of flannel fabric, sewn into 2 layers, on which circles of filter paper were placed. The pads were washed and boiled in water for 30 min after each placement. Immediately before seed placement, tissue linings were sterilized by boiling for 10 min. The germination bed (filter paper) was moistened immediately prior to seed placement by with distilled water. Then, 100 seeds were taken from pure seeds isolated after purity determination and treatment with microwave radiation. Seeds prepared for germination were laid out on a bed of filter paper in 25 pieces using tweezers previously sterilized by boiling for 10 min. A constant bed temperature (22±2°C) was provided by heating and maintaining the water temperature inside the seed germination apparatus at 24°C daily for 24 h. Constant temperature (22±2°C) was supported on a bed with seeds, through a filter from the bed, water came from a tray with water in which the water temperature was maintained at 24°C. The seed germination bed (filter paper) was moistened immediately before the seeds were laid out.

The bed and water temperatures in the machine were checked at 8 a.m., 12 p.m., and 6 p.m. During seed germination, illumination was provided for 8 h. The evaluation and registration of germinated seeds were carried out daily. The first day of germination was considered the day following the day of nesting. The end of germination was considered the last day of the seed germination record. On the day of each recording of seedlings, normally germinated and decayed seeds were removed from the bed and the number of seeds was noted in the analysis card, separately for each sample: normally germinated, decayed, and non-germinated seeds left on the bed. Before removing seeds from each bed, the tip of the tweezers was wiped with a cotton swab dipped in alcohol. On the day of the final recording of germination, the seeds remaining on the bed were opened along the embryo, separately for each sample, and the number of healthy, abnormally germinated, rotten, steamed, embryonic and empty seeds infected with pests was determined. The data obtained was recorded into the analysis card.

During the isoenzyme analysis, 6 seeds were used for each enzyme system. According to the cleavage of allozyme variants of any locus in the endosperms of the seeds of this tree, the individual's heterozygosity was judged by this locus. The research was conducted in All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology (Voronezh, Russia).

To prepare the extracts, the seeds were soaked in distilled water a day before analysis. Before analysis, the peel was removed from the seeds, and the embryo was separated from the endosperm. Each endosperm, and, if necessary, each embryo was placed in a separate homogenizer tube and homogenized separately. 0.1 ml of the extraction buffer was added to the homogenizer; after complete grinding, the pestle was removed and washed with 0.1 ml of the same buffer. The test tube with the extract was left to infuse in the refrigerator for 1 h at +2-4°C. The entire contents of the homogenizer tube were used for analysis.

As an extraction buffer, we used Tris-HCl electrode buffer with the pH of 8.3, diluted in 5 times, to which a 40% sucrose solution was added in a 1:1 ratio. Tween-60 was dissolved in the buffer to a 1% concentration. The composition of the electrode buffer included 6.0 g of tris-(oxymethyl)-aminomethane and 28.8 g of glycine in 1000 ml of distilled water (pH=8.3).

Analytical electrophoresis was performed in polyacrylamide gel plates. The compared extracts were applied to the tracks of one plate. Electrophoresis was carried out in a refrigerator freezer at -15°C for 1.5-2 h until the mark (bromophenol blue) reached the end of the gel at a distance of 0.5 cm. Bromophenol blue was added to the upper electrode buffer before the start of electrophoresis. Electrophoresis was carried out first at 80 mA (200 V), then at 150 mA (350 V). After the end of electrophoresis, the gels were removed, washed with distilled water, and placed in a mixture for staining with the corresponding enzyme.

The composition of the solutions is shown in table 1. The solutions of the fine-pore gel included Sol.1 and Sol. 2. To prepare the gel, the solutions were mixed in a 1: 1 ratio. The composition of the large-pore gel solutions included Sol. 3 – Sol. 6. To prepare a large pore gel, the solutions were mixed in a ratio of 1: 2: 1: 4. All solutions were stored in a refrigerator.

For staining glutamate dehydrogenase, a solution consisting of: M Tris-HCl buffer pH=8.0 – 50 ml;
glutamic acid – 45 mg; Nicotinamide adenine dinucleotide (NAD) – 10 mg; Nitro blue tetrazolium (NBT) – 1 ml (2.5 % solution); Phenazine metosulfate (FMS) – 1 mg. was used. The gels were poured and incubated at +37 °C in the dark. The color appeared in the places of localization of the enzyme in the form of blue or brownish stripes (depending on the quality of the reagents).

Table 1. The solutions of the fine-pore and large-pore gel.

|                | Sol. 1 | Sol. 2 | Sol. 3 | Sol. 4 | Sol. 5 | Sol. 6 |
|----------------|--------|--------|--------|--------|--------|--------|
| Tris-(oxymethyl)-aminomethane, g | 9.15   | -      | 5.98   | -      | -      | -      |
| 1 M HCl, ml    | 12.0   | -      | 48.0   | -      | -      | -      |
| N, N, N’, N”-tetramethylethylenediamine, ml | 0.115  | -      | 0.46   | -      | -      | -      |
| Acrylamide, g   | 15.0   | -      | -      | 10.0   | -      | -      |
| N, N’-methylene-bis-acrylamide, g | 0.4    | -      | -      | 0.4    | -      | -      |
| Ammonium persulfate, g | -      | 0.14   | -      | -      | -      | -      |
| Riboflavin, mg  | -      | -      | -      | -      | 4.0    | -      |
| Sucrose, g      | -      | -      | -      | -      | 40.0   | -      |
| Distilled water pH 8.9, ml | < 100  | < 100  | -      | -      | -      | -      |
| Distilled water pH 6.7, ml | -      | -      | < 100  | < 100  | < 100  | < 100  |

The enzyme superoxide dismutase was detected during staining with glutamate dehydrogenase if, after filling the gel block with the reaction mixture, the cuvette was kept in the light for several minutes and then incubated in the dark at 37°C. In this case, after 1 h, the gel turned blue, on which white stripes appeared at the sites of superoxide dismutase localization.

Dry seeds of Pinus sylvestris L. were exposed to microwave radiation for 3, 4, and 5 min. Extracts of individual endosperms and embryos were subjected to electrophoretic separation. To identify the dynamics of changes in the isoenzyme spectra of seeds, after exposure to microwave radiation, endosperms and embryos were analyzed for 1-13 days. In the seeds previously soaked in distilled water, the skin was removed and the embryo was separated from the endosperm. They were then homogenized separately in homogenizer tubes with 0.1 ml of extraction buffer. Further, electrophoresis was performed in the polyacrylamide gel plates in the freezer at a temperature of –15 °C, for 1.5–2 hours, at which the compared extracts were applied to the tracks of one plate. After the end of electrophoresis, the gels were placed in a mixture for staining on the corresponding enzyme. A total of 46 definitions of glutamate dehydrogenase (GDG) and 46 definitions of superoxide dismutase (SOD) were performed.

Before carrying out the research, we made preliminary calculations to determine the objective optimization function – the radiation efficiency factor. The purpose of these calculations was to find a certain interval of variation of the irradiation dose \( G \), at which the efficiency factor takes the highest value. As a result of calculations, it was found that the highest efficiency coefficient is observed at the value of the irradiation dose \( (G) \) of 5 min. To conduct a comprehensive experiment, the following intervals of variation of the parameter \( G \) were chosen; the minimum value is 1 min, and the maximum value is 15 min.

Statistical processing of the research results was carried out using a computer with the STATGRAPHICS - Statistical Graphics System (Statistical Graphics System) application package, and was also processed by one-factor analysis using the STAT 1 program.

During the experiment, the germination rate and seed germination energy were taken into account. After experimental studies, the coefficients of the radiation efficiency \( W \) were calculated. The input factors were the radiation doses (exposure time) and the observation time. The output parameters were seed germination and \( W \) coefficient.

At the first stage of the research, the control and measurements of the accepted input factors and output parameters were carried out in accordance with the experimental procedure.

When processing the research results of all the obtained output data, the sample size was at least 40
observations.

Statistical analysis of the data obtained was carried out in four stages. At the first stage, primary statistical processing was carried out with the calculation of the main indicators of the sample.

At the second stage, the hypothesis of the normal distribution of the obtained data was tested. The specified check was carried out according to the Kolmogorov-Smirnov agreement criterion and the \( \chi^2 \) criterion. Calculations using the Kolmogorov-Smirnov goodness-of-fit test were performed for preliminary verification, and calculations using the \( \chi^2 \) test were performed to thoroughly test the hypothesis of normal distribution.

The third step was to determine correlation factors between the input and output parameters, and in calculating the coefficients of their mutual dependencies. At the same time, the correlation coefficient was calculated to determine the statistical relationship between the following values: efficiency coefficient (seed germination), radiation dose (seedling height), efficiency coefficient (seedling height), and radiation dose (seed germination).

At the fourth stage, the significance of the obtained regression equations was checked. This check was carried out by comparing the calculated values of the Student's t-test with the permissible (tabular) values. The adequacy of the regression equations was established by Fisher's criterion.

The primary statistical processing of the obtained experimental results consisted in determining the following statistical characteristics: sample mean, median, mode, standard deviation, minimum, maximum, coefficient of variation. The data collected in the study of the dynamics of growth, germination is represented by a large number of numbers, which were brought into a certain system. This arrangement of observation materials is called a summary of statistics. As a result of a summary of statistical data (on the height of seedlings, seed germination) relative to one statistical value (on doses of treatment), distribution series, or variation series, were obtained. After creating the variation series, we began to calculate its numerical characteristics (indicators). Power-law averages are the most widely used in mathematical statistics. The main statistic al indicator of the distribution series is the average of the first degree, i.e. arithmetic mean, or mean.

3. Results and discussion

3.1. Germination and energy of germination of seeds Pinus sylvestris L.

As a result of the calculations, it was found that the highest efficiency coefficient is observed at the value of the microwave radiation doses \( G = 5 \) min.

The average indicators of germination and germination energy vary by samples (table 2). At a very high germination rate of seeds with Pinus sylvestris L. treated (in stimulating doses) with ultrahigh-frequency radiation for 5 min (77%), the indicator of germination energy was also significant even for 5 days. The germination duration is 126 h (5.3 days) when the seeds are treated with ultra-high-frequency radiation for 5 min on day 12, all the seeds have sprouted.

Table 2. Laboratory germination/germination energy of Pinus sylvestris L. seeds after exposure to microwave radiation, %.

| Exposure time, min | 1   | 3   | 4   | 5   | 7   | 10  | \( \geq 12 \) |
|-------------------|-----|-----|-----|-----|-----|-----|-------------|
| Germination rate, %| 68  | 69  | 74  | 77  | 46  | 0   | -           |
| Seed germination energy, %| 59  | 61  | 67  | 69  | 43  | 0   | -           |

According to the results of the experiment, stimulating, depressing and lethal doses were observed. Seeds treated with stimulating doses were further examined in the open ground to measure ground germination, growth and development of seedlings. The negative effect on the seeds of Pinus sylvestris L. was distinguished by the treatment with microwave irradiation at an exposure of 7 and 10 min. With an increase in these doses, a fatal outcome occurred in the exposure of 12 min.

For seeds of Pinus sylvestris L. treated in stimulating doses, a statistical analysis of the data was performed (table 3). The control version of the seeds is characterized by lower germination rates. On the seventh day, the seeds began to germinate slightly, on the tenth their germination rate averaged
45%, and in 15 days – 67% with an average germination time of 266 h (11.1 days).

The higher the seed germination, the lower the level of variation of the indicator. So, when treating seeds with ultrahigh-frequency radiation for 5 min, it was equal to 3.1, 32.5 and 9.4 %, respectively, for 12, 5 and 7 days, while in the control version, for 15 days – 21.3 % and for 10 days – 45.6 %. Thus, the variability of germination by day (cumulative total) by the end of germination is reduced.

The germination of seeds after exposure to physical factors in stimulating doses was higher than the standard (at least 75 % [14]) for the II quality class, while the control was of the III class (at least 65 %).

| The period of germination, days | Correlation coefficient and regression | Relative error, % |
|-------------------------------|---------------------------------------|------------------|
|                               | $R_{\pm S_y}$ | $t_r$ | $y=ax+b$ | $S_{ex}$ |                      |
| Ultrahigh-frequency radiation exposure of 5 min |                       |       |         |            |                      |
| 5                             | 0.24±0.343      | 0.65  | -       | -        | -                 |
| 7                             | 0.77±0.226      | 2.70  | 0.29x+71.2 | 2.2 | 2.2   |
| 10                            | 0.79±0.217      | 2.83  | 0.55x+44.9 | 2.1 | 2.1   |
| 12                            | 0.98±0.070      | 6.08  | 0.94x+6.2 | 0.7 | 0.7   |
| Control                       |                       |       |         |            |                      |
| 7                             | 0.12±0.204      | 0.70  | -       | -        | -                 |
| 10                            | 0.60±0.160      | 3.40  | 0.71x+69.4 | 14.3 | 19.0  |
| 15                            | 0.98±0.073      | 14.06 | 0.95x+11.2 | 4.2 | 5.6   |

Other indicators have seeds treated in depressing doses: germination of class III quality [14], the duration of the germination period is 18 days, the course of germination does not have a clearly pronounced increase in any day, as was observed in other variants, but goes more evenly. Germination here began only on the 12th day, and according to the existing method at the forest seed stations, they would be classified as non-standard in germination.

In general, the dynamics of seed germination under different types of irradiation is very similar, while maintaining significant differences in germination and seed dormancy duration. Radiation exposure at different doses also had a different effect on the seeds. Perhaps this explains a certain progressive state – a significant deviation from the usual course of germination. It is possible that the quality of the seeds will be affected by the size of the crop: the higher it is, the better the germination rate, and vice versa. The seeds of *Pinus sylvestris* L. were harvested in a year of weak harvest.

Between the indicators of germination by day (cumulative total) and at the end of the germination period, there is a positive correlation of different levels, but in all cases its straightness is statistically significant ($F_r > F_{0.05}$, where $F_r$ and $F_{0.05}$ – accordingly, the actual and theoretical Fisher criterion for significance 5%). Seeds of *Pinus sylvestris* L., treated with ultrahigh-frequency radiation for 5 minutes, in the initial period (4-5 days), their germination is higher, but the level of correlation at the end of the period is very low and unreliable. Here, on the 5th day, the correlation coefficient increases to 0.24, but with a given number of observations, it does not reach statistical reliability. On the 7th day, the correlation level increases significantly, becomes reliable, and the equation error is only 2%. By the 12th day, it decreases to 0.7 % with the correlation coefficient 0.98±0.07.

It should be noted that, despite the duration of exposure, the correlation coefficient indices germination energy and germination of seeds is almost the same and equals 0.77…0.79.

To better characterize the germination of seeds, we have calculated the relative germination rate, where control was taken as 100% (figure 1). In the graphs shown that the maximum germination was in seeds of *Pinus sylvestris* L., processed in exposure 5 min – 119%.
Figure 1. Relative germination of Pinus sylvestris L. seeds in laboratory experiments after microwave irradiation.

As a result, the positive effect of microwave irradiation on the germination of Pinus sylvestris L. seeds in an exposure of up to 5 min was proved, with an increase in the treatment time, the germination and germination energy decrease up to a lethal outcome.

3.2. Isoenzyme analysis of the effect of microwave radiation on Pinus sylvestris L. seeds at the cellular and subcellular levels

In our studies, microwave radiation with a frequency of 2.45 GHz was used to stimulate the germination of Pinus sylvestris L. seeds. The following results were obtained: compared to the control, seed germination increased by 10-15% when exposed for 5 minutes compared to the control.

To explain the experimental data obtained, we will use the studies of the authors of [15-18], who considered the interaction of radio frequency radiation with biological objects at the same frequency that was used in our research.

The results obtained allowed us to characterize the changes occurring in the EMR field at the levels of the protein and lipid phases, as well as in the region of lipid-protein interactions, more precisely, at the lipid-water boundary.

The first phase of the membranes was studied using two methods: by the fluorescence intensity of the natural protein label-tryptophan [19] and by the circular dichroism method [20, 21]. In fluorimetric studies, the maximum radiated UPM was equal to 200 W/kg, the heating of the suspension during exposure in a thermostatically controlled cell was 3.4°C (initial temperature 33.6°C, duration of exposure 5 min.)

It was found that structural changes occur in proteins under the influence of the EMR field, accompanied by an increase in the availability of tryptophan residues for water. Qualitatively and quantitatively, they corresponded to the heating observed when the samples were heated to the same temperature using a thermostat.

Studies using the circular dichroism method were carried out at higher UPM: from 150 to 725 W/kg. They corresponded to an increase in temperature by 1.8...5.8°C. Irradiation in a temperature-controlled cell, the initial temperature is 32°C, the exposure is 10 min. The structural rearrangement of proteins in the EMR field detected by this method only coincided qualitatively with that caused by conventional heating. In quantitative terms, these actions were significantly more effective.

A number of fluorescent probes were used to study the lipid phase: diphenylhexatriene [22], pyrene, and perylene. The locations of these probes in the membrane vary. All probes showed an increase in their mobility (a decrease in the viscosity of the microenvironment) as the temperature increases due to EMR absorption. In parallel, the extinguishing of pyrene intensified. Similar changes occurred during the temperature control. Within the limits of the experimental error, the effects of
EMR and heating were not significantly different, although there was a tendency for EMR to be more effective.

Basic information about the state of the lipid-water contact area (lipid-protein interactions) in the EMR field was obtained using naphthalene sulfone probes of 1.8-aniline naphthalene sulfonate 2.6-toluidinone naphthalene linsulfonate. The difference between these probes lies in the different orientation of the long axis of the probe molecule in the polar region of the lipid bilayer, that is, the different depth of immersion of the probes in the lipid bilayer. The conditions of the experiments were similar to those with pyrene and perylene.

It was found that there were changes in the localization of the probes under the influence of the EMR field, accompanied by a decrease in the constant of their binding to the membrane, and they were similar to those caused by heating. In quantitative terms, there were no statistically significant differences between the samples heated by EMR and the thermostat, although in the case of EMR, there was a tendency to their greater efficiency.

Thus, it can be stated that under the influence of EMR, structural changes occur in proteins that lead to their activity and are accompanied by an increase in the availability of tryptophan residues for water.

Let us consider the features of the influence of the studied physical factors on the enzyme systems of glutamate dehydrogenase (GDH) and superoxide dismutase (SOD), using the results presented in table 3. The greatest impact on the enzyme system of GDG was caused by the irradiation of seeds with microwave radiation in the exposure of 5 minutes. In dry seeds, the changed spectra appeared on day 2-3, and on Day 8 after treatment, after the normalization of the spectra, their repeated change was observed (table 4). When processing wet seeds, the changes in the spectra occurred faster and lasted for a longer time, the return to normal did not occur even on Day 13.

**Table 4.** Changes in the electrophoretic spectra of enzymes after exposure to microwave radiation on wet and dry seeds *Pinus sylvestris* L.

| Days of observation | Seed treatment time, min |
|---------------------|--------------------------|
|                     | 3                        | 4                        | 5                        |
| Glutamate Dehydrogenase | norm                    | norm                    | norm a single doubling of the spectra of the endosperm |
| 1                   | norm                     |                         |                          |
| 3                   | norm                     |                         |                          |
| 4                   | norm                     |                         |                          |
| 5                   |                          |                         | a partial increase in the activity of increased activity |
| 8                   |                          |                         | reduced activity, the blurring of the spectra, the germ activity higher than that of the endosperm |
| Superoxide dismutase |                          | the increase of activity |                         |
| 1                   |                          | the increase of activity |                         |
| 3                   |                          |                         | increased activity       |
| 4                   |                          |                         |                            |
| 5                   |                          |                         |                            |
| 8                   |                          |                         |                            |

Irradiation of the seeds with microwave led to an immediate increase in the activity of SOD in all treatment options. Seeds treated for a longer time retained the increased activity of the enzyme for longer. By the 8th day after the last normalization, the variants with 3 and 4 min of exposure showed a decrease in SOD activity lower than in the control. The variant with 5 min of irradiation passed into the phase of attenuation of activity, also, possibly, through the phase of normalization, which,
however, could not be recorded due to a two-day break in the analysis (the analysis was carried out on the 5th and 8th days after treatment).

The study of the nature of changes occurring in the spectra of glutamate dehydrogenase showed that this enzyme responds to the effects of radiation in stimulating doses by a violation (rearrangement) of its quaternary structure, leading to the appearance of additional bands in the isoenzyme spectrum (figure 2) and a change in their mobility. Since the GDH molecule is considered [19] to have a complex quaternary structure, representing a hexamer, the impact can cause its partial destruction with subsequent compensation, leading to the reversibility of the initially observed effects. The rearrangement of the quaternary structure of the GDG can obviously be considered a non-specific reaction to various kinds of stressful influences.

![Figure 2. Isoenzyme spectra of glutamate dehydrogenase in normal (a) and after microwave irradiation (b); superoxide dismutase in normal (c) and after microwave irradiation – the disappearance of the activity of some and the decrease in the activity of other bands (d); E – endosperm, Z – embryo.](image)

The reaction of superoxide dismutase to stimulating radiation doses was, as a rule, an increase in activity without rearrangements of the isoenzyme spectra. At the same time, in almost all variants, after more or less prolonged stimulation, there is a slight drop in activity below the control and a subsequent return to normal. The change in the activity of SOD can obviously be considered a non-specific reaction to various kinds of stressful effects (figure 2).

In research papers [23-27], the nonthermal effects of electromagnetic fields interacting with biological systems have been studied. It is shown that the use of optical and dielectric spectroscopy methods is promising for explaining the mechanism of interaction of EMR with biological systems. Models have been developed in which semi-quantitative calculations are presented. In these calculations, the focus was on the observation of a gene under the influence of electromagnetic fields.

The works of the authors [28-33], in which the microwave field was used as a stimulating effect of carrot, corn and other seeds, are of interest.

A microwave household oven with a power of 0.5 kW in the working chamber at a frequency of 2450 MHz was used as a microwave installation in [34]. Seed samples in paper bags were placed on a glass stand, at a height not lower than a quarter wavelength (4.0-4.5 cm) in the middle of the resonator chamber. The radiation dose was calculated indirectly from the water load (water poured into the flask and installed in the middle of the chamber). Doses from 20 to 120 J/kg were used in the work. The stimulating effect can be traced in the marketable yield at modes of 30-40 sec and reaches 26.2 %, and
the safety of root crops is 36.79%.

Corn seeds irradiation [35] was carried out using a typical signal generator G4-142 and a setup based on a magnetron of 8 mm radio wave range in laboratory conditions. A bell-shaped horn was used as an antenna. The seeds were irradiated in a Petri dish located in the plane of the horn opening. It provided the required level of power flux density of electromagnetic radiation. The study of plant growth and development was evaluated using generally accepted methods. The results of the study indicate that the nature of the effect of millimeter-wave electromagnetic radiation on the germination energy, field germination and the duration of the stages of organogenesis depends on the maize genotype, wavelength and exposure to radiation.

In recent years, theoretical studies have appeared on the effect of a strong microwave field on seeds. Thus, in [29] it was shown that the safety of grain in a microwave field is guaranteed, and thermal field dissociation makes it possible to qualitatively explain the inertial process of changing grain properties in strong microwave fields, and the material itself is not only a simple and effective absorber of electromagnetic waves, but also an interesting physical object.

N.F. Morozov patented a device for pre-sowing treatment of grain crops with a low-frequency electromagnetic field with signals of various shapes (rectangular shape, saw, sinusoid and their combinations) [36]. The field energy did not exceed the breaking energy of hydrogen bonds in the seed; and in all cases, an increase in yield was obtained by 20-25%. A device for microwave pre-sowing seed treatment is proposed by other authors [15]. They used microwave energy to irradiate seeds, in the millimeter and centimeter wavelength ranges, with high uniformity of treatment and high productivity.

Experiments in [16-18] have shown that irradiation of seed only with EMR of the centimeter wavelength range leads to the same results as irradiation of seed only with EMR of the millimeter wavelength range, namely, an increase in germination, an increase in the length of shoots and an intensification of growth. Roots and an increase in the final yield by 20% compared to unirradiated seed. The cumulative effect of millimeter and centimeter wavelength radiation on the planting material leads to a higher result - an increase in the final yield up to 67%.

In another series of studies [36], the presowing treatment of Pinus sylvestris L. seeds with corona discharge fields is considered. It has been found that soaking seeds in water for 5 hours and treatment with corona discharge fields with an exposure of 5 min increases the seed germination energy by 11…18.5%. The studied methods of pre-sowing preparation had an insignificant effect on seed germination. With a further increase in the time of soaking and the time of treatment with corona discharge fields of seeds, their sowing qualities approach the control values. In works [37, 38] the authors used pretreatment of seeds of Vicky vulgaris (Vicia sativa L.), Chrysanthemum Latirus (Lathyrus chrysanthus Boiss.) with radioactive radiation (low gamma radiation, radioactive cobalt (60Co) γ-rays) separately or in combination with salt stress and drought stress resulted in a significant increase (p < 0.01) in dry matter accumulation, CAT, SOD and APX activity, proline content, and a decrease in relative water content. Overall, these results showed that pretreatment with low doses of gamma radiation can increase the seedlings' tolerance to salt and drought stress.

Processing of microwave irradiation pine seeds also causes stress factor, which tracked in the study of the enzyme system, so that the seeds had higher vigor and germination. Over time, after treatment, the stress factor leveled off, the stimulating effect has a limited duration. In the study of seedlings in the open field, a higher germination capacity and germination energy were revealed, but the growth of seedlings did not exceed the control (not treated) variant.

4. Conclusion
As a result of the studies and statistical processing of the data, it was found that during the pre-sowing treatment of seeds with microwave irradiation in an exposure of up to 5 minutes, a gradual stimulation of germination occurs, and starting from 7 minutes, a sharp decrease, up to a lethal outcome. Exposure to stimulating doses of microwave irradiation in an exposure of 5 minutes on the seeds of Pinus sylvestris L. maximally increased to 79% compared to 65% for control. A high indicator of seed
germination energy indicates the appropriate germination rate for the entire germination period. Laboratory studies have shown that seed treatment in stimulating doses reliably affects germination and germination energy, causing physiological processes that both accelerate and slow down seed germination. The exploratory studies carried out revealed a complex picture of the manifestation of effects after exposure to different types of radiation on the enzyme systems of glutamate dehydrogenase and superoxide dismutase of endosperm and seed embryos of Pinus sylvestris L. The specificity of enzyme systems on stress effects was revealed, the dynamics of their change in the period after irradiation is ambiguous and depends on the time of irradiation. In general, seed treatment with stimulating doses causes reversible changes in the structure and function of enzymes. When exposed to stress factors (radiation), an increase in the activity of enzymes is observed, leading to the excitation or inhibition of growth processes at the first stages of development. As a result, a stimulating effect arises – the germination of seeds increases, the growth of seedlings in height increases, or depressing – the germination decreases and the growth of seedlings slows down. In the future, the activity of enzymes is stabilized, which leads to the normalization of physiological processes, therefore, the restoration of the normal growth of seedlings.

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