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Effect of hydrostatic pressure on seed germination and subsequent growth of *Silybum marianum* (*L.*) Gaertn. seedlings in Middle Ural introduction

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Abstract. The influence of pre-sowing treatment of holy thistle seeds (Silybum marianum) by hydrostatic pressure (from 10 to 200 MPa) on the dynamics of the emergence of seedlings and the subsequent development of seedlings in the open ground in the Middle Urals has been studied. It is shown that the treatment of seeds with a pressure of 10 MPa during their subsequent sowing a day later (after drying) provided good germination and accelerated the passage of all stages of the ontogenesis of holy thistle plants in the dry growing season of 2021. At the same time, earlier and more friendly maturation of seeds was observed in comparison with the control group of plants. Under more favourable weather conditions in 2022, holy thistle seeds after the same pressure treatment (10MPa) and drying showed very good field germination (76%), a high rate of ontogenesis of seedlings similar to the reference and the best development of generative structures.

1 Introduction

Recently, attention to the study of the influence of hydrostatic pressure (as an external factor) on living organisms has been growing. It is known that this factor, especially at its high levels, has a significant impact on living organisms in natural conditions. For example, it is a powerful environmental factor in relation to the inhabitants of the deep sea [1]. Hydrostatic pressure is also used for various purposes as an artificial factor of influence on biological objects, including plant seeds [2]. In relation to plants of different families, studies were conducted to assess the germination and germination dynamics of seeds treated with hydrostatic pressure. Several publications have noted the positive and negative effects of such treatment on the germination of alfalfa seeds, sweet clover, vigna, watercress, etc. [3-6]. At the same time, some works indicate certain physiological and biochemical changes in the cells and tissues of seed germs and seedlings. Thus, when

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processing brown rice with a pressure of 30-90 MPa for 5 minutes, embryo growth slows down and the total amount of digestible and stable starch decreases [7]. Flour from pressure-treated millet fruits has improved functional properties [8]. In connection with the effect of hydrostatic pressure, the long-range transport of solutions in the body was studied [9]. During cytological studies, it was found that one of the targets of the influence of hydrostatic pressure on plant cells is the endomembrane system [10]. Thus, studies of the effect of hydrostatic pressure on different structures and parts of the plant organism are promising. Regarding economically valuable plant species, it is relevant to study the influence of this factor on seeds, their biological properties and the subsequent development of plants. The results of such studies can become the basis for the development of technological methods to increase production and improve the quality of plant raw materials.

In our work, the object of research was holy thistle *Silybum marianum* (*L.*) Gaertn., sem. Asteraceae. It is a valuable medicinal plant grown in many countries [11, 12]. Many biological, ecological, and biochemical features of this plant have been well studied, which opens prospects for experimental ecological studies of holy thistle as a model object.

The homeland of holy thistle is the Mediterranean. The northern and eastern parts of the natural range cover the southern regions of the European part of Russia, the south of Western Siberia, many areas of the Caucasus and Central Asia. It does not form natural thickets. Industrial blanks are possible only during cultivation. Holy thistle is cultivated as an annual, medicinal plant raw materials are fruits (seeds). They contain a complex of flavolignans, which have hepatoprotective properties. Holy thistle in culture is characterized by significant morphological and physiological variability, its seeds ripen well. However, the technology of cultivation of this plant in the conditions of the Middle Ural for obtaining high-quality medicinal raw materials is difficult. Ways to improve this technology are being sought [13].

We assumed that exposure to high hydrostatic pressure on holy thistle seeds would cause positive changes in plant development. At the same time, we can expect an increase in the yield and an increase in the quality of medicinal raw materials of this plant.

2 Materials and methods

The treatment of holy thistle seeds by hydrostatic pressure was carried out using a laboratory hydrostat. Industrial oil was used as a transmission medium. Sealed elastic containers filled with water were used to exclude the contact of oil and seeds. Before processing, the seeds were placed in containers, then the containers were dipped in oil. A detailed description of the equipment used and high hydrostatic pressure treatment details is given in [14]. Holy thistle seeds were treated once at pressures from 10 to 200 MPa for five minutes with a sharp pressure drop. After the treatment, seeds were removed from containers and dried with filter paper. Further, these seeds were used to conduct field experiments on the territory of the Botanical Garden of the Ural Branch of RAS (Middle Ural, Yekaterinburg) in 2021 and 2022 in the period from May to October.

A prepared plot with homogeneous soil was selected for sowing seeds. The influence of various variants of pre-sowing seed treatment by hydrostatic pressure on the emergence of seed seedlings and on the subsequent development of holy thistle plants was evaluated. The seed samples used for the 2021 experiments were collected in 2020 from plants obtained as a result of introduction in the botanical garden. 360 seeds were treated with different levels of hydrostatic pressure (from 10 to 200 MPa). They were divided into groups for the following experience options: 10 MPa, 25 MPa, 50 MPa, 100 MPa, 200 MPa and reference sample (without pressure treatment). In each experimental variant, 60 seeds were exposed to the appropriate pressure level.

In 2022, seeds obtained in the same introduced population in 2021 were used in the field experiment. A random sample of 315 seeds was formed, which were treated with different levels of hydrostatic pressure in an aqueous medium in the range from 5 to 20 MPa for 5 and 10 minutes, while the seeds swelled. Reference sample seeds were placed in water for a time corresponding to the stay of seeds in water during pressure treatment. Further, all seeds were planted in the open ground immediately after the high-pressure treatment, apart from the variant in which, after the high-pressure treatment (10 MPa, 5 min), the seeds were dried at room temperature and sown the next day. Thus, the following variants of the experiment were formed: 20 MPa, 5 min; 10 MPa, 5 min; 10 MPa, 10 min; 5 MPa, 5 min; reference sample; 15 MPa, 5 min; 10 MPa, 5 min with drying.

Sowing in 2021 was carried out one day after the high-pressure treatment and drying of seeds on May 27th, and in 2022 immediately after the high-pressure treatment on May 30th on plots of 1 m^2 (6 plots for each variant of the experiment in 2021, 5 plots in 2022), the seeding rate is 9 seeds per 1 m^2. The distance between the plants in the rows is 30 cm, in the aisles 50 cm. The emergence of seedlings was considered, the timing of the onset of the main phenophases in plant development, their morphological characteristics at different stages of ontogenesis (in each of the five ontogenetic states) were noted throughout the growing season. To describe ontogenesis, the concept of the discrete nature of individual plant development was used [15, 16]. The obtained data were processed using Excel computer programs.

3 Results

In a field experiment with ground crops of holy thistle seeds treated with different levels of hydrostatic pressure, the following results were obtained.

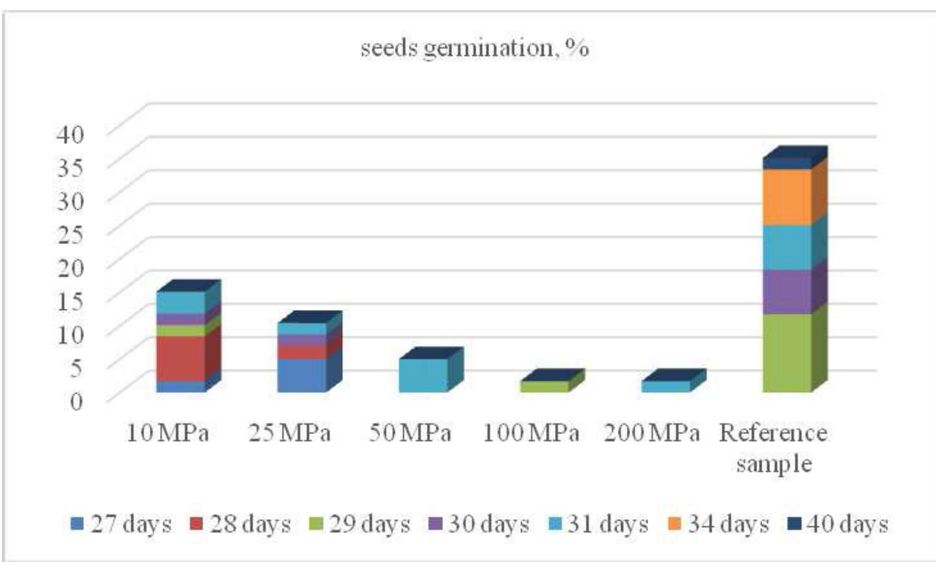

Fig. 1. Dynamics of the emergence of holy thistle seedlings according to the variants of the experiment in the period from 27th to 40st day after sowing in 2021.

As can be seen from Figure 1, the maximum field germination in 2021 of holy thistle seeds (33%) was observed in the reference sample, 20% – in the variant with pre-sowing seed treatment with a pressure of 10 MPa, 15% – in the variant with higher pressure treatment (25 MPa). Seeds exposed to higher levels of hydrostatic pressure (50, 100 and 200 MPa) showed very low field germination (Figure 1).

The bar charts in Figure 1 shows significant differences between the variants of the experiment in the dynamics of the emergence of holy thistle seedlings. Sowing was carried out on May 27th, 2021, and the first sprouts appeared only on the 27th day after sowing, and exclusively only in variants with pressure treatment at 10 MPa and 25 MPa. For the first time, such a prolonged period of emergence of holy thistle seedlings was noted. Most likely, this is due to the abnormally hot weather in May and June, and the minimum amount of precipitation in the Middle Ural in 2021. Thus, the average temperatures in May and June reached 18.5°C and 19.3°C, respectively, which is significantly higher than the average monthly temperatures over the past 7 years. The amount of precipitation was 16 mm and 52 mm, respectively, which indicates a significant drought in the territory of the Yekaterinburg city. In this regard, watering of the experimental plot was regularly carried out, but it did not have a positive effect on the speed of seed germination. In the reference variant, the first, but friendly shoots (the maximum daily number of seedlings in this variant) appeared only on the 29th day after sowing. Seedlings in the variants 100 MPa and 200 MPa appeared on 29-31st day. It should be noted that the seeds in the reference sample germinated within 3 days, the seeds with pressure treatment at 10 MPa germinated within 5 days, at 25 MPa - within 4 days after the start of germination in the experiment as a whole.

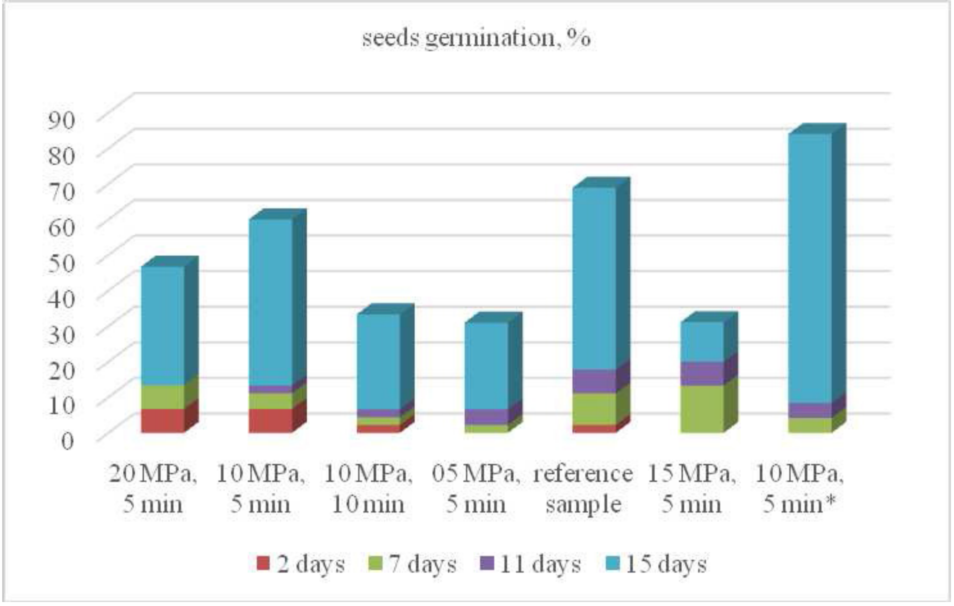

Fig. 2. Dynamics of the emergence of holy thistle seedlings according to the variants of the experiment in the period from 2nd to 15th day after sowing in 2022.

In 2022, the first seedlings appeared in four groups (20 MPa, 5 min; 10 MPa, 5 min; 10 MPa, 10 min; reference sample) on the second day. On the 7th day, seedlings appeared in all the studied groups. On the 15th day, the maximum number of seedlings was in the group of 10 MPa, 5 min with drying (76%), 69% - in the reference variant, 60% - in the variant of 10 MPa, 5 min, 47% - in the variant of 20 MPa, 5 min, 33% - in the variant of 10 MPa, 10 min, 31% - in variants 5 MPa, 5 min and 15 MPa, 5 min (Figure 2).

Thus, in 2021, the maximum number of seedlings appeared in the reference group, and in 2022 – in the variant of 10 MPa, 5 min with pre-drying.

The next stage of the work was to assess the passage of the main stages of ontogenesis and phenological phases throughout the growing season. It is known that the complete ontogenesis of *S. marianum* plants in the Middle Ural takes place in one growing season [13]. Five ontogenetic states were identified: seedlings (p), juveniles (j), immatures (im), virginal (v) and generative (g) plants [15]. In the experiment conducted in the hot and dry summer of 2021, the first sprouts (seedlings) appeared on 27-31st days after sowing, while under standard weather conditions in the Sverdlovsk region, according to the results of experiments of previous years, shoots appeared no later than on the 16th day after sowing. So, in 2022, the first shoots appeared on the 2nd day after sowing.

Seedlings (p) bear two cotyledons, which are rounded at first, and then turn into obovate. The first real leaves appeared in plants in the variant with 10 MPa pressure treatment on the third day, in the reference group – on the fourth day, in all other variants of the experiment – on the sixth day. The first real leaves form within 13-15 days, after which the plants pass into a juvenile state.

Juvenile plants (j) are rosette shoots with two true obovate leaves. The largest leaves were formed in plants in variants of 10 MPa and 25 MPa, they had a length (on average) of 1.6 cm and 2 cm, respectively. In the reference group, such leaves reached a length of 0.6 cm, which is two to three times less than in the compared groups with high pressure treatment.

Immature plants (im) on a rosette shoot bear 4-6 leafless leaves of an obovate shape. It should be noted that 4-5 true leaves appeared massively in plants in variants of 10 MPa and 25 MPa on 15-16 days after germination, while in the reference group 6 days later. The length of the leaf blade varies in plants of the 10 MPa variant in the range of 5-12 cm, in

Table 1. Phenological phases of *Silybum marianum* (*L.*) Gaertn. in culture in the Middle Ural (2021-2022).

Treatment	Sprouts	Vegetative phase	Budding (start)	Flowering (start)	Fruiting (start)
2021					
10 MPa, 5 min	22.06	28.06-1.08	02.08	9.08	26.08
25 MPa, 5 min	22.06	28.06-8.08	09.08	30.08	10.09
50 MPa, 5 min	28.06	05.07-8.08	09.08	30.08	no
100 MPa, 5 min	28.06	28.06-8.08	09.08	30.08	10.09
200 MPa, 5 min	28.06	05.07-8.08	09.08	30.08	no
Reference	24.06	28.06-8.08	09.08	12.08	2.09
2022					
20 MPa, 5 min	01.06	06.06	18.07	25.07	16.08
10 MPa, 5 min	01.06	14.06	11.07	18.07	16.08
10 MPa, 10 min	01.06	14.06	11.07	18.07	16.08
5 MPa, 5 min	06.06	14.06	18.07	25.07	16.08
Reference	01.06	06.06	11.07	18.07	16.08
15 MPa, 5 min	06.06	06.06	11.07	18.07	16.08
10 MPa, 5 min*	06.06	10.06	18.07	25.07	16.08

*- dried

plants of the 25 MPa variant – from 4 to 12 cm, and in the reference sample – 3.5-10 cm. That is, immature holy thistle plants formed from seeds after pressure treatment in the 10 MPa and 25 MPa variants have larger leaves than in the reference group.

Virginal plants (v) have large and long leaves. In the experiment conducted at this stage, the reference plants are leafier compared to the other groups. Reference plants are more voluminous, juicy and bright.

Next comes the generative period (g), when the budding phase is recorded, then flowering and fruiting. The timing of the passage of the main phenological phases in holy thistle plants according to the variants of the experiment is indicated in Table 1. From the data in Table 1 and Fig. 1, in 2021, with the most uniform and friendly appearance of seedlings in the reference group, these plants recorded a later transition to the phases of budding and flowering compared to the 10 MPa variant. At the same time, the plants in the reference group formed very large, well-leafed. However, by October 1, most of these plants did not have time to complete the fruiting phase: in many inflorescences, the seeds did not reach a mature state. Plants managed to completely go through all the stages of ontogenesis in the variant with 10 MPa seed pressure treatment. They passed all the phenophases faster and since the end of August they have been bearing fruit massively. At the end of September, inflorescences with ripe fruits were collected from them.

In 2022, a week earlier, all phases of plant ontogenesis took place in the variants of the experiment 10 MPa, 5 min, 10 MPa, 10 min, reference group. Plants in the variant of 15 MPa, 5 min rose later, but at the same time entered the flowering phase with the variants of early shoots. Plants in variants of 20 MPa, 5 min, 5 MPa, 5 min, 10 MPa, 5 min with drying entered the flowering phase later than all other plants, but at the same time all experimental variants entered the fruiting phase in the same way.

4 Discussion

According to the results of 2021, it can be concluded that in order to obtain a large volume of phytomass for agricultural use, it is better to use the seeds of *S. marianum* of the reference group, which showed good mass field germination during the dry growing season. To obtain medicinal plant raw materials – the fruits of *S. marianum*, it is better to use seeds with a pressure treatment of 10 MPa for sowing, since these plants go through all stages of ontogenesis faster and bear fruit massively, unlike reference group.

In 2022, all phases of plant ontogenesis of all experimental variants took place much earlier than in 2021. Plants of experimental variants under pressure treatment of 10 MPa (5 min and 10 min) and 15 MPa passed all stages of ontogenesis in the same way as in the reference group, unlike 20 MPa, 5 MPa and 10 MPa during drying, but all plants simultaneously entered the fruiting stage. Thus, this experiment shows that in order to obtain friendly shoots and maximum fruiting, seeds with an exposure of 10 MPa, 5 minutes with drying should be used. This variant of pressure treatment showed positive results during the two years of the study. Plants of this variant can adapt to the extremely hot weather conditions of the Middle Ural (2021) by accelerating the passage of all phases of ontogenesis and obtaining mature fruits. And in moderate weather conditions (2022) these plants showed high field germination and the passage of all stages of ontogenesis almost on a par with the reference plants.

This research has practical significance both for agricultural, biological, pharmaceutical disciplines, and for physical sciences. When using hydrostatic pressure seed treatment technologies, positive results can be obtained: an increase in plant productivity, a reduction in the timing of their ontogenesis, an acceleration of seed germination, and consequently an increase in the raw materials obtained.

5 Conclusion

Consequently, to obtain medicinal plant materials (fruits) of holy thistle, it is advisable to use seeds for sowing after treatment with a hydrostatic pressure of 10 MPa, followed by drying and sowing in a day.

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