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# Germination and Seed Viability of *Helichrysum maracandicum* Popov Ex Kirp. Sterilized under *in Vitro* Conditions

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## Abstract

Most medicinal plants are on the verge of extinction. In this regard, biotechnology is facing the challenge of developing alternative ways to produce biomass with the desired Biological active substance. The maximum yield of high growth performance of the cell culture mainly depends on the selection of optimal ratios and concentrations of growth regulators. This problem, namely the search for the optimal composition of the nutrient medium has become one of the main tasks in the cultivation of *H. maracandicum* Popov ex Kirp plant cells. Methods and results of seed sterilization of *H. maracandicum* are discussed in the article. This endemic, rare species of medicinal plant from the flora of Uzbekistan family Asteraceae Dumotr. has a unique composition of secondary metabolites. For example, from the biomass of immortelle were isolated flavonoids, coumarins, lipids, phenols, purines, steroids, triterpenoids, glycosides, coumarins, cerines, bitter tannins, essential oils, etc. Used in folk medicine for cholecystitis and diseases of the liver, bladder, and gastrointestinal tract.

## Keywords

*Helichrysum maracandicum*, *in Vitro*, Inoculum, Sterilization, Seedling Viability

## 1. Introduction

Plant resources are of importance for agriculture, medicine, and ornamental gardening, but the potential of the beneficial properties of many plants has not

yet been sufficiently studied and is of interest in the future. Methodological approaches to the study of collections are based on the principle of maximum coverage of genetic diversity, including wild species, introduced plants, as well as the collection fund of plants cultivated *in vitro*.

One promising approach is the method of cultured cells *in vitro* based on somatic cells [1]. Until that time, the authors have studied the bioecological features and cultivation of some rare and introduced medicinal plants in various conditions of Uzbekistan [2] [3] [4].

At present, much experience has been accumulated in *vitro* propagation of rare, rare medicinal plants [5] [6] [7] [8] [9], as well as obtaining useful metabolites from the biomass of their vegetative and generative organs [10] [11] [12]. At the same time, relevant studies on growing *H. maracandicum* plants from their tissues and organs in vitro have not been conducted. For this purpose, the germination and viability of *H. maracandicum* seeds under in vitro conditions were studied at this stage of the study. According to the literature, the effect of in vitro sterilization of *H. maracandicum* seeds on their fertility and seedling viability has not been studied by anyone before in Samarkand or Uzbekistan. In addition, information on seed germination of species of the genus *Helichrysum Mill*. is poorly reflected in the literature. For example, according to Khujanov [13], the highest germination of *H. maracandicum* seeds in laboratory conditions was 82% within 30 days at 25˚C, while the data of this author provide no information on seedling viability.

It is known that seed germination biology includes multifactorial processes of exogenous (temperature, humidity, light, storage conditions) and endogenous (structure of seed coat, physiological state during germination) parameters. According to these factors, exogenous, endogenous, and combined resting are distinguished in plant seeds [14].

## 2. Object and Methods of Research

### 2.1. Seeds Collection and Sorting.

Seeds of plants were collected from the natural population of *H. maracandicum* in the vicinity of Kyzylbisoy village (Amankutan, Urgut district, Samarkand region, in the northern part of Zeravshan range; 66˚89'03.83"E 39˚29'75.93"N) [13]. Soil is grey soil mixed with fine sand. According to A. N. Khujanov, the mass of 1000 dry seeds of *H. maracandicum* is 0.08 ± 0.01 g in size, prismatic-brown seeds, called seedpods, have a length of 1.3 - 2 mm and a width of 0.2 - 0.6 mm (**Figure 1**). One of the unique characteristics of these seeds is their ability to maintain germination for up to 3 years [13]. It is known that the study of seed fertility of plants is one of the main criteria when restoring natural populations of plants and creating their plantations. As the period of seed storage increases, their germinating capacity decreases.

The Germination of *H. maracandicum* seeds was studied according to Ishmuratov's method [14], and their germination capacity was determined according to

Figure 1. Selection of seeds of helichrysum maracandicum popov ex kirp.

Firsova's method [15]. According to the literature, the germination of freshly harvested seeds is higher when growing medicinal plants from their seeds [10]. Well-matured annual seeds of *H. maracandicum* were selected for our studies (Figure 1).

### 2.2. Seed Sterilization.

The method of R. G. Butenko [16] was used for the sterilization of plant tissues and organs. To remove pathogenic microflora, seeds were subjected to surface sterilization with tap water and then distilled water at room temperature. The following seed sterilization scheme was used to remove the internal symbiotic infection, which has a harmful effect when growing seeds in vitro.

To prepare 1 liter of alkaline Domestose solution used for sterilization, 50 ml of Domestose solution was added to 950 ml of water. The antibiotic streptomycin was used at a concentration of 330 μg/ml. After each reagent treatment, the seeds were washed with sterile distilled water.

## 3. Results Obtained and Their Analysis

### Germination of Sterilized Seeds and Seedling Viability

Several different sterilization methods were tried to sterilize *in vitro* the seeds of *H. maracandicum*, which is considered the object of the study.

Consistently, the seeds were washed in water for 1 hour, shaken vigorously in a 1% solution of domestose (soapy water), washed 6 times in water, washed in a solution of the antibiotic streptomycin (330 μg/ml), washed in water 3 times, in 70% ethyl alcohol, then washed while shaking for 1 minute, shaken thoroughly, and washed 3 times in clean water. The medium and conditions of sterilization are given in the table below (Table 1).

Based on this scheme, four variants of sterilization were studied. In the first variant, the seeds were shaken intensively in 1% domestose solution for 5 minutes, and the antibiotic streptomycin solution was kept for 6 minutes. In the second variant, the seeds were shaken in 3% domestose solution for 5 minutes and kept for 8 minutes in the antibiotic solution of streptomycin. After treatment with ethyl alcohol, the seeds were washed with intensive shaking for 1.5 min. In the third variant, the seeds were shaken in a 5% solution of domestose

**Table 1.** Stages and variants of in vitro sterilization of *H. maracandicum* seeds.

| № | Stages of sterilization | Sterilization options | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| 1 | Water | 60 min. | 60 min. | 60 min. | 60 min. |
| 2 | Domestose | 1%, 3 min. | 3%, 5 min. | 5%, 8 min. | 8%, 10 min. |
| 3 | Water | 6 times | 6 times | 6 times | 6 times |
| 4 | Streptomycin (330 μg/ml) | 6 min. | 8 min. | 10 min. | 12 min. |
| 5 | Water | 3 times | 3 times | 3 times | 3 times |
| 6 | Ethyl alcohol (70%) | 1 min. | 1.5 min. | 2 min. | 2.5 min. |
| 7 | Water | 3 times | 3 times | 3 times | 3 times |

for 8 minutes, and in the solution of the antibiotic streptomycin was kept for 10 minutes. The last rinsing with water was carried out under intensive shaking for 2 minutes. In the fourth variant, the seeds were shaken for 10 minutes in 10% domestose solution and incubated in the streptomycin antibiotic solution for 12 minutes. The last rinsing with water was carried out under intensive shaking for 2.5 minutes. As can be seen from the table, the variants used in the experiment differed in the number of stages of cross-sterilization and their duration.

According to A. N. Khujanov, the optimum germination temperature of *H. maracandicum* seeds in laboratory conditions is 25˚C, and the germination rate is 80% - 82% [10]. The germination of seeds that passed the sterilization stages in the above variants was studied for 30 days in the medium with an air temperature of 25˚C. The results of our studies are shown in **Figure 2(a)**. We can see that seed germination was 23% in the first sterilization option, 31% in the second, 96% in the third, and 16% in the fourth. That is, the highest germination rate observed in the third variant of seed sterilization, seed germination was 14% higher than that noted by A. N. Khujanov [10].

Observations of the viability of seedlings obtained from seeds after seed sterilization were carried out for 15 days. Analysis of the results of observations of seedling viability at the level of 10%, 15% and 90% was noted in variants 1, 2 and 3. In contrast, in the fourth variant, 16% of the lawns remained on the 5th day of observations, and by the 10th day, all the seedlings died (**Figure 2(b)**).

## 4. Conclusions

The results (**Figure 3**) obtained showed that for in vitro studies with *H. maracandicum* the most effective sterilization of *H. maracandicum* seeds, was used in variant 3. Contrary to the other variants, in this case, we used to wash the seeds for 8 minutes with 5% domestose solution, keeping them for 10 minutes in a streptomycin solution, and then for 2.5 minutes in 70% ethanol. Under these conditions, the germination of *H. maracandicum* seeds reached 96%, and the

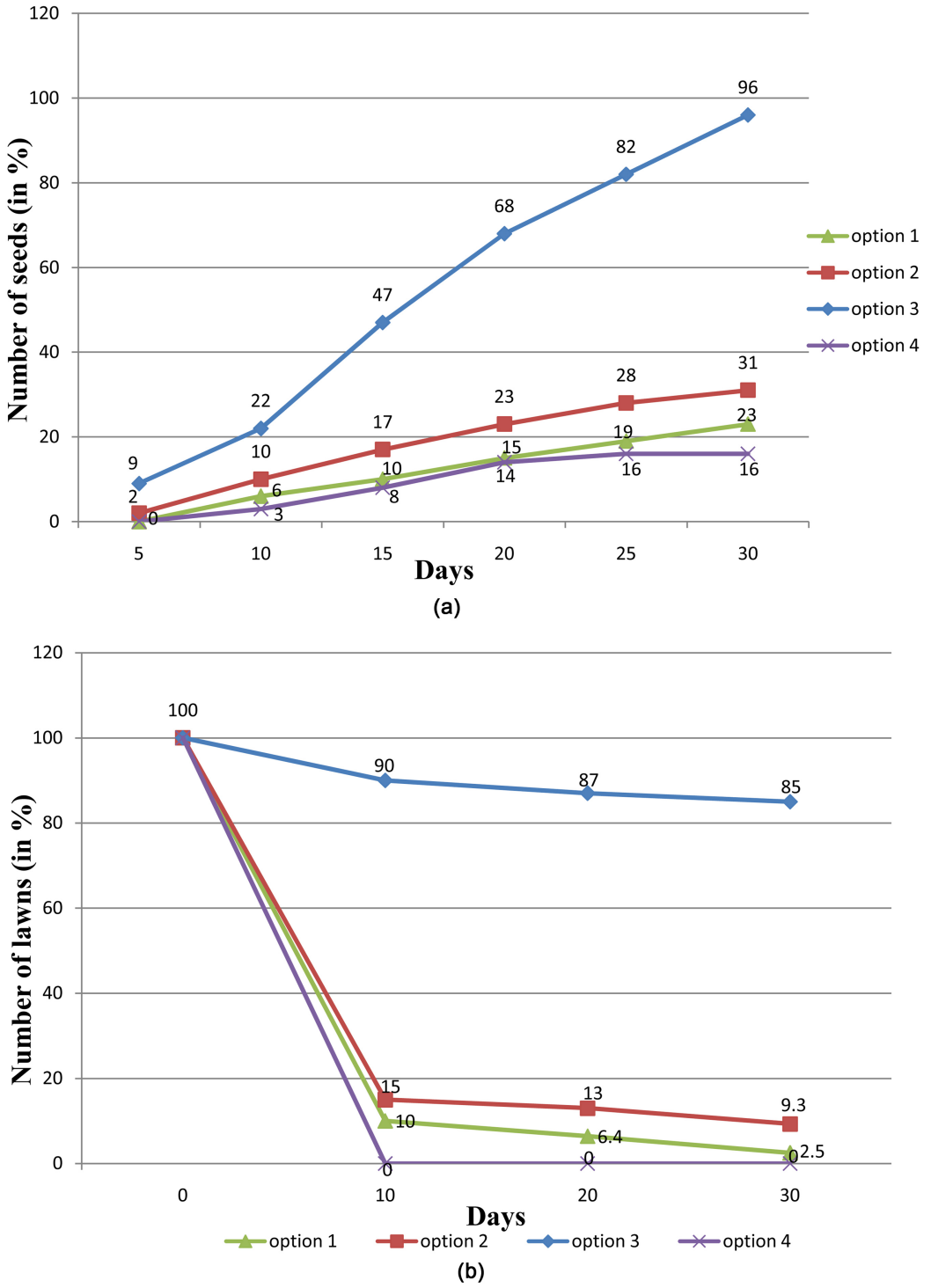


**Figure 2.** Germination of *H. maracandicum* seeds (a) and seedling viability index (b) after different types of sterilization. Option, sterilization options (see **Table 1**).

viability of the prostrates, 85%.

Thus, the proposed scheme of sterilization of seeds of *H. maracandicum* Popov ex Kirp allows us to obtain a high yield of viable seedlings of this species to obtain cells and tissues for further work on cell engineering.

**Figure 3.** *H. maracandicum* seedlings.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## Fund

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