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INTRODUCTION OF *DRACOCEPHALUM MOLDAVICA L. IN VITRO* CULTURE

Background. ***Dracocephalum moldavica L. is widely used in folk medicine in many countries of the world due to its antispasmodic, antibacterial, analgesic, sedative and anti-inflammatory properties. D. moldavica essential oil is often used in aromatherapy and cosmetology. The use of biotechnological methods for obtaining secondary plant metabolites has become increasingly popular in recent years. Onthe one hand, they serve as a source of substances beneficial for pharmacology, while on the other hand, aseptic microclones of medicinal plants can be utilized as model organisms to explore methods to enhance the biosynthesis of specific target products. The in vitro use of plants for the production of biologically active substances is also a crucial and pertinent method for the conservation of natural biodiversity. The aim of this study was to determine the optimal conditions for introducing the medicinal plant Dracocephalum moldavica into in vitro culture.***

Methods. ***To introduce Dracocephalum moldavica plants into in vitro culture, the conditions for seed sterilization were carefully selected. The effectiveness of treatment with succinic acid in combination with various concentrations of sodium hypochlorite solution (20 %, 50 %, or 70 %) was assessed to obtain aseptic material and ensure a high percentage of seed germination. The duration of seeds viability after storage for 1, 2, and 3 years was determined by testing seed germination in soil and in vitro.***

Results. ***Seeds of D. moldavica have a relatively low germination rate when planted in soil and quickly lose their viability. In vitro cultivation can significantly enhance the germination of fresh seeds and allow for a more efficient use of two- and three-year-old seeds. The stimulating effect of succinic acid on the seed germination and seedling development of Dracocephalum moldavica has been observed, but it also activated the growth of fungal and bacterial infections. Therefore, it is advisable to use this stimulant only along with a more intensive sterilizing agent.***

Conclusions. ***The optimal method for introducing Dracocephalum moldavica from seeds into in vitro culture has been determined: pre-treatment of seeds with succinic acid followed by sterilization using 70 % sodium hypochlorite.***

Keywords: *succinic acid, sterilization, seed viability, percentage of seed germination.*

Background

The increasing demand for herbal medicines in the healthcare sector highlights the importance of addressing the issue of securing a raw material base through additional plant sources and their integrated use. The widespread and uncontrolled exploitation of essential oil plants has resulted in the depletion of many raw materials, emphasizing the necessity to investigate alternatives that can ensure a sustainable raw material base. In this context, essential oil plants of the Lamiaceae family are particularly noteworthy. They contain a complex of biologically active substances, including essential oils, terpenoids, saponins, polysaccharides, phenolic compounds, etc. These plants exhibit versatile pharmacological activity and low toxicity, making them of significant interest (Gu, et al., 2004; Davazdahemami et al., 2022).

Dracocephalum moldavica L. (moldavian dragon, moldavian snakehead, garden pistil) is a native plant for the temperate climate of Asia. However, it is also found in eastern and central Europe. Over the years, *D. moldavica* has been used for a variety of purposes, including medicinal, cosmetic, culinary, and ornamental (Zhan et al., 2024).

The aerial parts of *D. moldavica* contain a range of compounds, such as phytoncides, flavonoids, phenolic carboxylic acids, and triterpenoids. The most well-known and studied components of *D. moldavica* are its volatile oils, responsible for its characteristic aroma and flavor (Rudy et al., 2020). In the aerial parts of *D. moldavica*, 23 compounds were identified in the essential oil. The primary components of the essential oil from the aerial parts of *D. moldavica* are oxygenated monoterpenes, including pulegone, menthone, and isomenthone. Other components of the volatile oil include limonene, β-pinene, trans-piperitone oxide, and 1,8-cineole (Lawless, 2013; Amini, et al., 2020).

The seeds of *D. moldavica* are a nutrient-rich source containing various bioactive compounds, including proteins, fatty acids, carbohydrates, minerals, and vitamins (Frąc et al., 2016). The primary fatty acids present in the seeds are linoleic acid (48.78 %), oleic acid (22.49 %), and palmitic acid (16.78 %) (Golparvar et al., 2016). Among the storage proteins in the seeds, vicillin and legumin were identified, which are commonly found in legumes. Additionally, the main protein fractions in the seeds of *D. moldavica* include globulin, albumin, prolamine, and glutelin.

The moldavian snakehead is extensively used in traditional medicine in many countries worldwide and is included in the pharmacopoeias of several European countries. Traditionally, *D. moldavica* is used in phytotherapy for various purposes. It has documented use as an antispasmodic (Martínez-Vázquez et al., 2012; Sadraei et al., 2015), analgesic (contains luteolin contributing to its sedative properties) (Golshani et al., 2003), analgesic (the main compound responsible for the analgesic effect is rosmarinic acid), antimicrobial effect (extracts is able to inhibit the growth of bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*) (Sonboli et al., 2005; Fallah et al., 2018; Aćimović et al., 2019; Cao et al., 2019). Additionally, it has demonstrated anti-inflammatory properties and contains eucalyptol, which stimulates the production of digestive enzymes.

The essential oil *of D. moldavica* is commonly used in aromatherapy for its pleasant aroma and calming and relaxing properties. It can be applied through various

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methods, including diffusers, massage oils, or added to bathwater.

Skin care: *D. moldavica* contains rosmarinic acid, which can kill gram-positive and gram-negative bacteria. Other compounds, such as flavonoids and essential oils, also exhibit antibacterial effects. This makes the plant potentially useful for treating skin conditions such as eczema, psoriasis, and acne. Rosmarinic acid's antioxidant properties help protect the skin from damage caused by free radicals, potentially reducing signs of aging (Dastmalchi et al., 2007). *Dracocephalum moldavica* L. essential oil contains various compounds that may benefit hair. For example, it contains limonene, which can strengthen hair and improve its structure. It also contains linalool, known for its soothing effect on the scalp, helping to reduce itching and irritation. Additionally, it has a pleasant scent that can be used to add natural fragrance to hair care products.

The use of biotechnological methods to produce secondary plant metabolites has gained increasing popularity in recent years. The use of *in vitro* plant cultures for the production of biologically active substances is also a crucial and relevant method for the conservation of natural biodiversity. Some studies on the *in vitro* cultivation of *Dracocephalum moldavica* plants have been conducted to increase the efficiency of obtaining useful metabolites from genetically modified plants. In particular, the plants were treated with *Agrobacterium*-mediated transformation to create hairy roots and subsequently obtain callus and suspension culture from them (Weremczuk-Jeżyna et al., 2013; Weremczuk-Jeżyna et al., 2017). The method of genetic modification is certainly effective in enhancing the synthesis of metabolites, but it is quite complex, and the resulting material is genetically unstable. At the same time, the method of sterilisation of the primary material for the introduction of these plants into *in vitro* culture, as described in the literature, proved to be ineffective (Weremczuk-Jeżyna et al., 2013).

The main objective: to identify the optimal conditions for introducing *D. moldavica* into *in vitro* culture. This will help, on the one hand, to obtain a source of substances useful for pharmacology, and on the other hand, *Dracocephalum moldavica* microclones can be used as model objects to identify new ways to enhance the biosynthesis of certain target products by selecting the culture medium, spectral composition of light, etc.

Methods

Two-year-old seeds were used as primary explants for the *in vitro* culture of *Dracocephalum moldavica* plants, as this is the best way to quickly obtain aseptic plants.The seeds were pre-washed with running water and distilled water. Subsequently, half of the seeds were further soaked for 1 hour in a 0.1 % succinic acid solution, while the other half remained untreated with succinic acid (Table 1). Due to the fact that *D. moldavica* seeds lose their germination rather quickly, and we used two-year-old seeds, it was advisable to stimulate their germination beforehand. To do this, we used treatment with succinic acid – an important intermediate product of plant metabolism, involved in the processes of cellular (Tricarboxylic acid cycle) respiration, which are activated during seed germination; also it is showed that seedlings obtained from seeds treated with succinic acid had a higher chlorophyll content, increased vegetative mass faster, had higher water use efficiency and demonstrated resistance to stress at the level of antioxidants (Singh, & Singh, 1972; Kiliç, 2023). Seed treatment was carried out prior to introduction into the culture, as to stimulate germination, seeds are treated with high concentrations of succinic acid for a short time, and to improve growth processes, much lower concentrations are used once. When added to the nutrient medium, the biostimulant will act for a very long time, and at high concentrations, it can adversely affect the development of seedlings.

Following the pretreatment, both the succinic acid-treated and untreated seeds were sterilized.The method of sterilisation of *D. moldavica* seeds described in the literature, using a 2 % sodium hypochlorite solution for 2 min (Weremczuk-Jeżyna et al., 2013), is very gentle and therefore ineffective. Therefore, for sterilisation, we used reagents that are traditionally used and have proven to be effective: higher concentrations of sodium hypochlorite solution and 0.02 % HgCl2 solution (Kushnir, & Sarnatska, 2005; Madhale, 2016). Such, all seeds were kept in 70 % ethyl alcohol for 1 minute, followed by a 10-minute exposure to a 0.02 % sulema solution and an additional 8 minutes in a sodium hypochlorite (NaClO) solution. Different concentrations of sodium hypochlorite solution were used for sterilization, specifically: 20 %, 50 %, or 70 %. Each experimental group consisted of 5 jars with 10 seeds in each. The details of the experimental groups are presented in Table 1.

Table 1

Experimental groups for treating *Dracocephalum moldavica* seeds with various solutions to stimulate germination and sterilization (n=50, M±m)

Variants of the experiment	I	II	III	IV	V	VI
Seed treatment with 0.1 % solution of succinic acid	+	+	+	-	-	-
Sodium hypochlorite concentration for seed treatment	20 %	50 %	70 %	20 %	50 %	70 %

After sterilization, the seeds were planted on Murashige and Skoog (MS) nutrient medium with half the concentration of substances (1/2 MS).

The experimental groups were monitored weekly to assess contamination presence and seed germination. The results of the sterilization efficiency were analyzed one month after planting to account for potential internal infections.

To determine the shelf life of seed viability, seeds from the first-year of harvest and those that had been stored for 2 and 3 years were selected. Germination took place in a greenhouse with a daytime temperature of +25 °C and a nighttime temperature of +20 °C, closely resembling optimal conditions for planting seeds in the ground. Ten seeds were placed in each well, with five wells per group.

For aseptic germination, seed sterilization followed a proven methodology from our previous study: 1 minute in 70 % ethyl alcohol, 10 minutes in sulema solution, and 8 minutes in 70 % NaClO.

Seeds with a shelf life of 1, 2, and 3 years were planted on ½ MS nutrient medium, with 10 seeds per jar and 5 jars per group.

The percentage of seed germination was calculated using formula (1) once the last individual seeds had germinated:

$$Germination\ \% = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds\ taken\ for\ germination} \times 100 \quad (1)$$

The obtained germination data were analyzed using the Prism Graphpad 8 program. The significance of the difference between germination values among groups with different sterilization methods, as well as with and without succinic acid pretreatment, was determined for each week separately using a multivariate ANOVA analysis with Tukey's correction. Differences at $p<0.05$ were considered significant.

Results

Sterilization of primary material

One week after planting on the nutrient medium, initial signs of fungal and bacterial infection emerged. Two weeks later, there was an increase in fungal contamination, possibly attributed to the germination of internal infections in the explants, with no further observable manifestations in the subsequent period. The results regarding the effectiveness of sodium hypochlorite in various concentrations, with or without pretreatment with succinic acid, are presented in Table 2.

Table 2

Amount of contaminated seed of *Dracocephalum moldavica* under different treatment with reagents

	I	II	III	IV	V	VI
Seeds treated with 0.1 % succinic acid	+	+	+	-	-	-
Sodium hypochlorite concentration for seed treatment	20 %	50 %	70 %	20 %	50 %	70 %
Amount of contaminated seed	80 %	60 %	20 %	40 %	40 %	20 %

According to the results of our studies, the most effective method for sterilizing *D. moldavica* seeds is to immerse them in a 70 % NaClO solution, both with and without pretreatment with succinic acid (group III). However, it is noteworthy that succinic acid treatment, in general, promoted the development of fungal and bacterial infection on the seed surface, resulting in the highest contamination rate (80 %) in the group with the least concentrated sterilizing agent (20 % NaClO). Increasing the concentration of NaClO to 50 % and 70 % reduced the infection of the primary material to 60 % and 20 %, respectively.

Germination of Dracocephalum moldavica seeds under in vitro conditions

Two-year-old seeds of *D. moldavica* in most experimental groups initiate *in vitro* germination 3 weeks after planting. Overall, a low germination percentage was observed, approximately 25 %, which could be attributed to the rapid loss of seed germination (Fig. 1). Seeds in experimental group V started to germinate only after 4 weeks, while in group I, no germination occurred. The latter can be explained by a significant fungal infection of the material (80 %) resulting from pre-sowing treatment with succinic acid. Additionally, a low germination percentage was noted in groups IV and V, where there was no seed stimulation with succinic acid, but due to insufficient sterilization, a high infection of explants was observed.

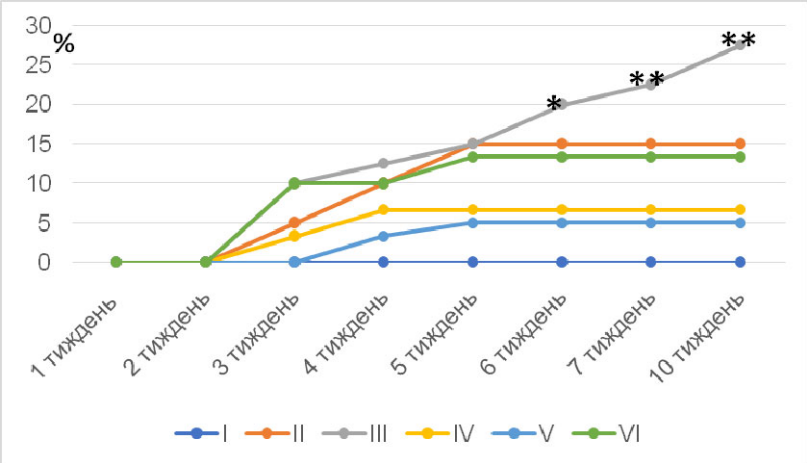

Fig. 1. Germination of two-year-old *D. moldavica* seeds depending on the different treatment with reagents. I – with succinic acid and 20 % NaClO, II – with succinic acid and 50 % NaClO, III – with succinic acid and 70 % NaClO, IV – without succinic acid with 20 % NaClO, V – without succinic acid with 50 % NaClO, VI – without succinic acid with 70 % NaClO

Note: * – significant difference ($p<0.05$) of group III compared to groups I and V after 6 weeks of germination;
** – a significant difference ($p<0.05$) of group III relative to groups I, IV and V after 7 and 10 weeks of germination

It should be noted that in most in vitro groups (II, IV, V, VI), seed germination ceased by the 5th week after seed planting. Whereas in group III, where seeds were pre-treated with succinic acid and sterilized with a high concentration of sodium hypochlorite, germination continued for up to 10 weeks. This group showed the highest germination rates as early as 6 weeks into seed cultivation (Fig. 1). Thus, the treatment of seeds with succinic acid stimulated germination,

while the high concentration of the sterilizing agent suppressed microorganisms activated by this acid on the seed surface. Additionally, in group III, the sprouts exhibited a thickened stem (Fig. 2). Simultaneously, in groups sterilized with lower concentrations of sodium hypochlorite (20 % and 50 %), after succinic acid treatment, there was an increase in fungal and bacterial infections, indicating the low efficacy of such sterilization. The intensified contamination, in turn, inhibited seed germination.

Fig. 2. *In vitro* plants of *D. moldavica* grown from seeds stimulated with succinic acid and sterilized in 70 % NaClO (group III)

In groups II and VI, the percentage of seed germination was average compared to other experimental groups. In group II, seeds and microorganisms were stimulated by succinic acid, but sterilization with 50 % NaClO was not effective. In group VI, sterilization with 70 % NaClO was successful, but there was no stimulating effect of succinic acid. Additionally, abnormal seedlings with shortened stems and low growth were found in both of these groups (Fig. 3).

Figure 1 demonstrates that under more optimal conditions (group III), two-year-old seeds continue to germinate for two months. This indicates a very low level of viability in the seeds, and they would not have germinated without additional stimulation and highly favorable conditions.

Statistical analysis of the results revealed significant differences between the groups only after 6 weeks of germination (Fig. 1). Thus, group III differed significantly from groups I and V after 6 weeks of germination, and after 7 and 10 weeks of germination, group III differed from groups I, IV, and V. The other groups did not differ significantly from each other.

Fig. 3. *In vitro D. moldavica* plants grown from seeds that were not stimulated with succinic acid and sterilized in 70 % NaClO (group VI)

Thus, the best result was obtained in group III: with pretreatment of seeds with succinic acid and subsequent sterilization using 70 % sodium hypochlorite. In this group, we found the least contamination, the highest germination rate, and the seedlings exhibited normal development with thicker stems.

Given the rather low germination capacity of the two-year-old seeds, even *in vitro*, the duration of seed viability was investigated, both *in vitro* and in soil. One-, two-, and three-year-old *D. moldavica* seeds were used. Fresh (one-year-old) seeds initiated active germination on the 6th day after planting in the soil, and by the 10th day, all viable seeds had germinated. Under aseptic conditions, fresh seeds began to germinate on the 5th day after planting and continued up to 14 days after planting. Thus, more favorable *in vitro* conditions, on the one hand, slightly accelerated the germination of viable seeds, and on the other hand, stimulated the germination of seeds with reduced viability (Table 3).

Table 3

Germination of *D. moldavica* seeds depending on its storage period and growth environment, % (n=100, M±m)

storage period / growth environment	1 year	2 year	3 year
In the soil	25±12,25	9±2,15	0±0
In vitro	98±2,74	28±3,56	6±0,34

Table 3 shows that fresh seeds of *D. moldavica* have a rather low germination rate when planted in soil (only 25% after one year of storage), when stored in dry conditions at room temperature, their viability decreases three times in the second year of storage and completely loses germination after three years of storage. When germinated *in vitro*, the germination rate of fresh seeds increases four times compared to those sown in soil, and that of two-year-old seeds increases three times, while only 6 % germinated after three years of storage.

Discussion and conclusions

D. moldavica seeds exhibit a rather low germination rate when planted in the ground and rapidly lose their viability. Therefore, we recommend using only annual seeds for planting in home gardens and greenhouses. Conversely, *in vitro* cultivation can significantly enhance the germination rate of fresh seeds, as well as more efficiently use two- and three-year-old seeds. By stimulating the seeds during cultivation on nutrient media and avoiding the negative impact of bacterial and fungal infections on seed germination, even seeds with low viability can successfully germinate. Hence, the duration of seed germination in aseptic conditions is notably longer than in soil.The nearly one hundred percent germination of fresh seeds is facilitated not only by the optimal composition of the nutrient medium and the appropriate humidity level in the crop but also by effective seed sterilization. One adaptive property of *D. moldavica* seeds is the intense secretion of mucilage by the seed coat after exposure to moisture. This mucilage helps the seed in retaining moisture under arid conditions in nature. However, this adaptation also potentially promotes the development of fungal contamination, which, in turn, can reduce the germination of weaker seeds.

An increase in the contamination of primary plant material was observed after pretreatment with succinic acid. Succinic acid, due to its properties, accelerates nutrient absorption, promotes plant growth, provides oxygen to cells, and has a protective function on living cultures. It is likely that pre-sowing treatment with this substance stimulated the development of bacterial and fungal infection on the surface of seeds before planting on the nutrient medium. Therefore, at low concentrations of disinfectants, the groups treated with succinic acid showed the highest contamination rates. A 70 % concentration of sodium hypochlorite proved to be the most effective in inhibiting the development of bacterial and fungal infections.

On the other hand, a stimulating effect of succinic acid was also observed on seed germination and seedling development of *D. moldavica*. Therefore, the most effective way to germinate seeds *in vitro* is to combine pre-sowing seed stimulation with succinic acid and sterilization with high concentrations of sodium hypochlorite. Plants introduced into *in vitro* culture can be further used directly for the production of valuable metabolites, or such aseptic plants can be stimulated to form callus, followed by the production of suspensions, which have also been shown to be effective for the production of valuable secondary metabolites (Weremczuk-Jeżyna et al., 2013; Weremczuk-Jeżyna et al., 2017).

Thus, in the course of our study, the optimal method for introducing *Dracocephalum moldavica* from seeds into *in vitro* culture was established: pretreatment of seeds for 1 hour with 0.1 % succinic acid and subsequent sterilization of seeds: 1 min. in 70 % ethyl alcohol, 10 min. in sulem, 8 min. in 70 % sodium hypochlorite.

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References

Aćimović, M., Sikora, V., Brdar-Jokanović, M., Kiprovski, B., Popović, V., Koren, A. & Puvača, N. (2019). *Dracocephalum moldavica*: Cultivation, chemical composition and biological activity. *J. Agron. Technol. Eng. Manag*., 2, 153–167.

Amini, R., Zafarani-Moattar, P., Shakiba, M.R., & Sarikhani, M.R. (2020). Essential oil yield and composition of Moldavian balm (*Dracocephalum moldavica* L.) as affected by inoculation treatments under drought stress conditions. *J. Essent. Oil-Bear. Plants*, 23, 728–742. https://doi.org/10.1080/0972060X.2020.1815593

Cao, W., Yuan, Y., Wang, Y., Tian, L., Wang, X., Xin, J., Wang, Y., Guo, X., & Qin, D. (2019). The mechanism study of *Dracocephalum moldavica* L. total flavonoids on apoptosis induced by myocardial ischemia/reperfusion injury in vivo and in vitro. *Biomed. J. Sci. Tech. Research.*, 20, 14985–14996. https://doi.org/10.26717/BJSTR.2019.20.003444

Dastmalchi, K., Dorman, H.J.D., Koşar, M., &Hiltunen, R. (2007). Chemical composition and in vitro antioxidant evaluation of a water-soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. *Journal of Food Science and Technolog*, 239–248. https://doi.org/10.1016/j.lwt.2005.09.019

Davazdahemami, S., & Allahdadi, M. (2022). Essential oil yield and composition of four annual plants (ajowan, dill, Moldavian balm and black cumin) under saline irrigation. *Food Ther. Health Care,* 4(1), 5. https://doi.org/10.53388/FTHC20220124005

Fallah, S., Rostaei, M., Lorigooini, Z., & Sukri, A.A. (2018). Chemical compositions of essential oil and antioxidant activity of dragonhead (*Dracocephalum moldavica*) in sole crop and dragonhead-soybean (Glycine max) intercropping system under organic manure and chemical fertilizers. *Ind. Crops Prod.* 115, 158–165.https://doi.org/10.1016/j.indcrop.2018.02.003

Frąc, M., Oszust, K., Kocira, A.,& Kocira, S. (2016). Molecular Identification of Fungi Isolated from *Dracocephalum moldavica* L. Seeds. *Agric. Agric. Sci. Procedia*, 74–79. https://doi.org/10.1016/j.aaspro.2015.12.036

Golshani, S., Karamkhani, F., Monsef-Esfehani, H. R., & Abdollahi, M. (2003).Antinociceptive effects of the essential oil of *Dracocephalum kotschyi* in the mouse writhing test. *Journal of Pharmacy & Pharmaceutical Sciences*, 6(2), 274–277.

Golparvar, A.R., Haidipanah, A., Gheisari, M.M., & Khaliliazar, R. (2016). Chemical constituents of essential oil of *Dracocephalum moldavica* L. and *Dracocephalum kotschyi* Boiss. from Iran. *Acta Agric. Slov*., 107, 25–31. https://doi.org/10.14720/aas.2016.107.1.03

Gu, H., Chen, R., Sun, Y., & Liu, F. (2004). Studies on chemical constituents from the herb of *Dracocephalum moldavica*. China *Journal of Chinese Materia Medica*, 29(3), 232–234.

Kiliç, T. (2023). Seed treatments with salicylic and succinic acid to mitigate drought stress in flowering kale cv. 'Red Pigeon F1', *Scientia Horticulturae*, 313, 111939. https://doi.org/10.1016/j.scienta.2023.111939

Kim, K.-M., Kim, S.-Y., Mony, T. J., Bae, H. J., Han, S.-D., Lee, E.-S., Choi, S.-H., Hong, S. H., Lee, S.-D., & Park, S. J. (2018). *Dracocephalum moldavica* Ethanol Extract Suppresses LPS-Induced Inflammatory Responses through Inhibition of the JNK/ERK/NF-κB Signaling Pathway and IL-6 Production in RAW 264.7 Macrophages and in Endotoxic-Treated Mice. *Journal of Medicinal Food,* 21(11), 1114–1123. https://doi: 10.3390/nu13124501

Kushnir, G.P., & Sarnatska, V. V. (2005). Microclonal reproduction of plants. *Naukova dumka*.

Lawless, J. (2013). The Encyclopedia of Essential Oils: The Complete Guide to the Use of Aromatic Oils in Aromatherapy, Herbalism, Health, and Well Being. *Conari Press*.

Madhale, S.V. (2016). Effect of HgCl2 on surface sterilization of explants of *M. cymbalaria* Hook. F. *J. Sci. Res. Int.,* 2(1), 39–43.

Martínez-Vázquez, M., Estrada-Reyes, R., Martínez-Laurrabaquio, A., López-Rubalcava, C., & Heinze, G. (2012). Neuropharmacological study of *Dracocephalum moldavica* L. (Lamiaceae) in mice: Sedative effect and chemical analysis of an aqueous extract. *Journal of Ethnopharmacology*, 139(2), 548–553. https://doi.org/10.1016/j.jep.2012.03.028

Rudy, S., Dziki, D., Biernacka, B., Krykowski, A., Rudy, M., Gawlik-Dziki, U., & Kachel, M. (2020). Drying characteristics of Dracocephalum moldavica leaves: Drying kinetics and physiochemical properties. *Processes*, 8, 509. https://doi.org/10.3390/pr8050509

Sadraei, H., Asghari, G., & Kasiri, F. (2015). Antispasmodic effect of Dracocephalum kotschyi hydroalcoholic extract on rat ileum contraction. *Research in pharmaceutical sciences*, 10(2),121–127.

Singh, S.,& Singh, K. (1972). Effect of seed treatment with succinic acid on the performance of cotton (Gossypium arboreum L.). *Indian Journal of Agricultural Sciences,* 42(2), 112–114.

Sonboli, A., Gholipour, A., & Yousefzadi, M. (2005). Antibacterial activity of the essential oil and main components of two *Dracocephalum*

species from Iran. *Natural product research,* 19(2), 155–159. https://doi.org/10.1080/14786419.2011.625501

Weremczuk-Jeżyna, I., Grzegorczyk-Karolak, I., Frydrych, B., Krolicka, A., & Wysokińska, H. (2013). Hairy roots of *Dracocephalum moldavica*: Rosmarinic acid content and antioxidant potential. *Acta Physiologiae Plantarum*, 35(7). https://doi.org/10.1007/s11738-013-1244-7

Weremczuk-Jeżyna, I., Grzegorczyk-Karolak, I., Frydrych, B., Hnatuszko-konka, K., Gerszberg, A.,& Wysokińska, H. (2017). Rosmarinic Acid Accumulation and Antioxidant Potential of *Dracocephalum moldavica* L. Cell Suspension Culture. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 45(1), 215–219. https://doi.org/10.15835/nbha45110728

Zhan, M., Ma, M., Mo, X., Zhang, Y., Li, T., Yang, Y., & Dong, L. (2024). Dracocephalum moldavica L.: An updated comprehensive review of its botany, traditional uses, phytochemistry, pharmacology, and application aspects. *Fitoterapia*, 172, 105732. https://doi.org/10.1016/j.fitote.2023.105732

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ВВЕДЕННЯ *DRACOCEPHALUM MOLDAVICA* L. В КУЛЬТУРУ *IN VITRO*

В с т у п . ***Dracocephalum moldavica L. широко застосовується в народній медицині багатьох країн світу завдяки наявності спазмолітичних, антибактеріальних, анальгетичних, седативних та протизапальних властивостей. Ефірна олія Dracocephalum moldavica часто використовується в ароматерапії та косметології. Застосування біотехнологічних методів отримання вторинних метаболітів рослин стає все більш популярним останніми роками. З одного боку, вони дозволяють отримати джерело корисних для фармакології речовин, а з іншого боку, мікроклони асептичних лікарських рослин можуть бути використані як модельні об'єкти для виявлення шляхів підсилення біосинтезу тих чи інших цільових продуктів. Використання рослин in vitro з метою отримання біологічно активних речовин є також одним з важливих і актуальних методів збереження природного біорізноманіття. Метою даної роботи було виявити оптимальні умови введення в культуру in vitro лікарської рослини Dracocephalum moldavica.***

М е т о д и . ***Для введення в культуру in vitro рослин Dracocephalum moldavica підбирали умови стерилізації насіння: перевіряли ефективність обробки бурштиновою кислотою в поєднанні з різними концентраціями розчину гіпохлориту натрію (20 %, 50 % або 70 %) з метою отримання асептичного матеріалу та високої схожості насіння. Визначали тривалість життєздатності насіння після зберігання 1, 2 і 3 роки шляхом перевірки схожості насіння у ґрунті та в умовах in vitro.***

Р е з у л ь т а т и . ***Насіння D. moldavica має досить низьку схожість при висіванні у ґрунт та швидко втрачає життєздатність, вирощування в культурі in vitro дозволяє значно збільшити проростання свіжого насіння, а також більш ефективно використовувати дво- та трирічне насіння. Виявлено стимулюючу дію бурштинової кислоти на проростання насіння та розвиток проростків Dracocephalum moldavica, але також при цьому спостерігалась активація росту грибкової та бактеріальної інфекції. Тому доцільно використовувати цей стимулятор лише поряд з більш інтенсивним впливом стерилізуючої речовини.***

В и с н о в к и . ***Встановлено оптимальний метод введення в культуру in vitro Dracocephalum moldavica з насіння: попередня обробка насіння бурштиновою кислотою та подальша стерилізація з використанням 70 % гіпохлориту натрію.***

К л ю ч о в і с л о в а : ***бурштинова кислота, стерилізація, життєздатність насіння, відсоток схожості насіння.***

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