Mathematical modeling of an experiment for the growth of chia seeds sprouts (Salvia hispanica L.)

Nadtochii L.A.¹, Venkatakrishnan K.², Jing J.³, Chechetkina A.Yu.¹, Muradova M.B.¹

¹ITMO University, Russia
²School of Nutrition, Chung Shan Medical University, 110, Sec. 1, Jianguo North Road, Taichung City, Taiwan, ROC
³Harbin Institute of Technology, China

* E-mail: l_tochka@itmo.ru

Abstract. World Health Organization (WHO) has reported that more than 80% of the population in developing countries uses herbal medicine. Modern technologies open up the possibility of controlling the cultivation of medicinal plants for commercial use. The combination of various methods of controlled cultivation is a high-tech and capital-intensive direction for the development of the agricultural industry in Russia. This article considers the selection of optimal conditions for growing of chia seeds sprouts as a multifactor experiment with three incoming factors at two levels. For this study 100 grains of dark varieties of chia seeds (Salvia hispanica L.) purchased from Era Green were used. The 3 major incoming factors include moisture (3, 4 and 5 ml of added water), the temperature (20, 25 and 30 °C), and the light (expose duration for 0, 12 and 24 h) under LED lamps with peaks at 440-450 nm (blue spectrum) and 660 nm (red spectrum). Using different factors, we found that the speed of germination and the seedling vigor were considerably improved. This multifactor model helps to establish more reliable information on the rational ways for obtaining chia seeds sprouts in the conditions of controlled cultivation with high yield.

1. Introduction
Currently, due to the decreasing effectiveness of synthetic drugs and increasing contraindications to their use, it is becoming relevant to use natural phytomedicines. Modern science has recognized its active effect and has included a number of herbal preparations known to ancient civilizations and used for millennia in modern pharmacotherapy [1].

According to statistics from the World Health Organization (WHO) has reported that more than 80% of the population in developing countries uses herbal medicine to treat various ailments. Moreover, herbal medicines usage in developed (industrial) countries is also growing in recent times for example, 25% of the UK population regularly takes herbal medicines [2]. In Europe, about 70-80% of total medicinal plants are collected from the wild [2] and the remaining are cultivated under controlled conditions for commercial purposes [3].

Collection of medicinal plants in the wild has a negative impact, such as reducing the number of plant species, the loss of genetic diversity, species of plants and habitat degradation [2]. In the case of wild harvesting, the constant selection of the largest wild-growing medicinal plants, which are considered desirable from a medical point of view, entails the destruction of the plant or the degradation of the wild population [4]. At present, 4,000 to 10,000 medicinal plant species are...
threatened with extinction [2]. Commercial use of medicinal plants inevitably affects the change in characteristics of plant populations. It is obvious that adequate protection of wild medicinal plants can be achieved by strengthening regulation by the state and introducing sustainable methods of plant collection. The long-term alternative in solving this problem may be by the development of the controlled cultivation of medicinal plants for commercial purposes, in order to optimize productivity and achieve uniformity of species and a high-quality product at the exit for pharmaceutical use [5].

The use of modern technologies for the cultivation of medicinal plants opens up the possibility of solving a number of biotechnological problems inherent in the production of herbal medicines, such as erroneous identification of species, their genetic and phenotypic variability, toxicity of plant components through environmental pollutants, as well as the instability of extracts based on them as sources of biologically active substances (BAS) [6, 7, 8]. As is known, BAS often are secondary metabolites of plants, which often serve as adaptations to changing factors such as fluctuations in temperature and light (for example, antioxidants), stress levels (for example, proline), infections (for example, flavonoids) and the consequences of eating plants by animals (for example, alkaloids) [9, 10, 11]. Scientists have discovered that the Atropa belladonna has been grown in the Caucasus has increased alkaloid content (1.3% compared to 0.3% of Sweden grown Atropa belladonna) [12]. Mentha piperita (peppermint) grown in shade has a lower content of essential oil (1.09% versus 1.43%) and menthol in oil (57.5% versus 61.8%) compared with Mentha piperita grown in the light. Papaver somniferum grown under cold conditions contains a higher morphine content but has a lower alkaloid content than Papaver somniferum grown in warm conditions [12]. The accumulation of secondary metabolites is also affected by the quantitative and qualitative composition of water in the soil, variations in pH and nutrients in the soil, as well as soil microorganisms [13, 14, 15].

The combination of various methods of controlled cultivation is a high-tech and capital-intensive direction for the development of the agricultural industry [16, 17]. Currently, commercial cultivation of plants can be carried out in open and/or close ground conditions (growing plants in a greenhouse), as well as using the hydroponic method (growing plants in special devices without soil) [18, 19]. It was revealed that the hydroponic method of growing plants in comparison with growing in the soil provides a quicker production of healthier plants, less susceptible to various diseases, etc. [20]. The hydroponic method of growing plants is a method of multifactorial impact on an object, taking into account on the control of air, room temperature, light, water, plant nutrition, and adverse climate, etc. [21].

There is a hydroponic method for growing plants from seeds (Pub. No.: US 2017/0150684 A1). The present invention uses one or more artificial light sources, such as light-emitting diodes (LEDs), designed to stimulate the growth and development of a plant by emitting an electromagnetic spectrum suitable for photosynthesis. This method provides various stages of plant growth at different densities of the photosynthetic photon flux at specially adjusted spectra ratios, preferably red, green, and blue, whereas yellow light effects considered less preferred. Although plants absorb green light to a lesser extent, a green spectrum of about 500 to 600 nm can be used at the stages of plant vegetation to enhance the green colour of a plant. Also, the duration of exposure to light is an important parameter affecting the growth and development of plants. According to this method, it is especially important to carry out at different densities of the photosynthetic photon flux for different phases of plant growth. The authors have presented various examples of preferred ratios between different light spectra, however, this indicator requires detailing regarding a single plant.

Recently, our group published a research paper, which reveals the optimal conditions for chia seeds sprouts [21]. The studies were carried out according to GOST 12038-84 ‘Agricultural seeds. Methods for determination of germination’, 100 pieces of Spanish sage were used as the test sample. It was found that the amount of added water, air temperature and exposure to light affect the production of Spanish sage sprouts. At the next stage of this study, it is necessary to take into account all of the above factors, which require the use of the multi-factorial design of the experiment.
2. Materials and Methods

Any effects on the research objects can be considered with respect to the theory of the control system. Figure 1 shows the structure of the experimental design taking into account incoming and outgoing factors. The selection of optimal conditions for growing of chia seeds sprouts is considered as a multifactor experiment, where the incoming factors are the temperature, moisture, and light factor, the outgoing factors are the speed of germination and the seedling vigor.

Mathematical modeling of an experiment is based on an analysis of data results from previous studies [21]. The values of the incoming factors which provide the values of the outgoing parameters with optimal performance are determined, thus the zero (main) background level is indicated. A value of an incoming factor at the zero levels (the level of "background") is denoted as $X_{i0}$, $X_{10}$, $X_{20}$, $X_{30}$ and so on. Then, the levels of change of a zero background are determined, as a rule, two levels are selected - upper and lower. For each factor, these levels differ from the main level (the initial level of the "background") by one and the same value, denoted as an interval of variation $\lambda_i$. As a rule, an interval of variation for each factor are different, which are selected on the basis of preliminary research data. Upper level and lower level $X$ are formed by adding or subtracting interval of variation (equations 1, 2).

\[
X^+ = X_{i0} + \lambda_i \quad (1)
\]
\[
X^- = X_{i0} - \lambda_i \quad (2)
\]

Thus, $X_{i0}$ represents an arithmetic mean between the selected levels of factors (equation 3).

\[
X_{i0} = \frac{X^+ + X^-}{2} \quad (3)
\]
To simplify the calculation of regression coefficients, the conversion (coding) of variable factors is carried out according to equations 4.

\[ x_i^{(+)} = \frac{x_i^{(-)} + x_i^{(0)}}{2} \]  

(4)

Where \( x \) – factor value of an upper or lower level in physical units; \( x \) – factor value in coded units.

In accordance with equation 4, the factors in coded units will be equal to +1 and –1. A number of experiment variants (u) depend on the number of incoming factors and is calculated by the formula \( m^n \). In total, at two levels (m) for three factors (n), for example, the number of experiment variants will be \( 2^3 \), equal to 8. This approach can be applied for a planned experiment by using an orthogonal matrix (table 1).

**Table 1.** Experiment planning matrix for three factors at two levels

| u  | \( x_1 \) | \( x_2 \) | \( x_3 \) |
|----|----------|----------|----------|
| 1  | +        | +        | +        |
| 2  | +        | +        | -        |
| 3  | +        | -        | -        |
| 4  | -        | -        | -        |
| 5  | -        | +        | +        |
| 6  | -        | -        | +        |
| 7  | -        | +        | -        |
| 8  | +        | -        | +        |

3. Results and Discussion

Based on previous studies [21] of incoming and outgoing factors, the experimental results are presented in Table 2.

**Table 2.** The results of chia seeds sprouts under the influence of incoming factors

| Incoming factors, per 100 grains of chia seeds | Outgoing factor, per100 grains of chia seeds |
|-----------------------------------------------|---------------------------------------------|
|                                               | the speed of germination, days               | the seedling vigor, grains                   |
| The moisture factor, ml of added water         | 3                                           | 4.0                                          | 12.9                                          |
| 4                                             | 5.2                                         | 13.1                                         |
| 5                                             | 3.8                                         | 12.9                                         |
| The temperature factor, °C                    | 20                                          | 5.2                                          | 12.6                                          |
| 25                                            | 5.3                                         | 13.1                                         |
| 30                                            | 5.2                                         | 9.7                                          |
| The light factor, duration, hours             | 0                                           | 5.2                                          | 13.0                                          |
| 12                                            | 5.2                                         | 13.0                                         |
| 24                                            | 5.3                                         | 13.0                                         |

The incoming factors of the experiment were 3 parameters, in particular, the moisture factor (ml of added water), the temperature factor (°C), the light factor (duration, hours) under LED lamp with
peaks at 440-450 nm (blue spectrum) and 660 nm (red spectrum). The outgoing factors of the experiment were 2 parameters: the speed of germination (days) and the seedling vigor (grains).

The outcome of the current study revealed that the outgoing factors (speed of germination/seedling vigor) are significantly affected by temperature and moisture factors. However, the influence of the light factor on chia seeds sprouts should be detailed in relation to different durations of exposure to light and its qualitative characteristics, specifically the spectral composition of light. Thus, an orthogonal matrix was compiled in order to optimize the process of obtaining chia seeds sprouts in a hydroponic installation (Table 3).

Table 3. Matrix of experiment planning for three factors at two levels with a different interval of variation

|   | $x_1,$ ml | $x_2,$ °C | $x_3,$ hours |
|---|-----------|-----------|--------------|
| 1 | 5         | 30        | 24           |
| 2 | 5         | 30        | 0            |
| 3 | 5         | 20        | 0            |
| 4 | 3         | 20        | 0            |
| 5 | 3         | 30        | 24           |
| 6 | 3         | 20        | 24           |
| 7 | 3         | 30        | 0            |
| 8 | 5         | 20        | 24           |

Three factors at two levels of variation were identified. As the changing factor 1 ($x_1$) on a zero background, the mass fraction of added water equal to 4 ml / 100 seeds and the interval of variation equal to 1 ml was chosen. As the changing factor 2 ($x_2$) on a zero background, the temperature equal to 25 °C and the interval of variation equal to 5 °C were chosen. As the variable factor 3 ($x_3$) on a zero background, the duration of exposure to light with using an LED lamp for 12 hours and the interval of variation equal 12 hours were selected.

The further formulation of the multifactor experiment according to table 3 will allow us to establish more reliable information on the rational ways for obtaining chia seeds sprouts in the conditions of controlled cultivation.

Figure 2 shows the scheme of interaction of the research object with the external environment, and the management principle of its state, which can be used to obtain chia seeds sprouts in controlled conditions. Impact on an object (P) is carried out using a control system (C) under certain conditions of planned exposure ($S_{n^*}$). In an ideal situation, certain conditions for planned exposure ($S_{n^*}$) and actual exposure ($S_n$) should be equal $S_{n^*} = S_n$. The reason for the violation of this condition may be interference from the environment or the reason for the limitation of control resources, which is expressed by the quadratic deviation according to the equation: $\Delta S_n = S_n - S_{n^*}$. The quality criterion of the process should be estimated using the vector of functionals $\varepsilon$, which is calculated by equation 5 [22]. Management based on this quality criterion is called controlled, which is important to consider for the growing of chia seeds sprouts in hydroponic conditions.

$$\varepsilon = \sum_{n} \sum_{r} \gamma_n [ (S_{n_r} - S_{n_r^*}) - (S_{n_{r-1}} - S_{n_{r-1}^*})]^2$$

Where the coefficient $\gamma_n$ is used to eliminate differences in the dimension of heterogeneous components $S_n$. 
4. Conclusions

Thus, taking into account the multifactoriality research object at the level of incoming and outgoing parameters the determination of the optimal factors for growing chia seeds sprouts under controlled conditions requires the detailed design of the experiment. This approach allows the researcher to establish a mathematical model for the experiment with the goal of the material and informational description of the studied object. To regulate the control system, it is proposed to use a quality criterion based on an estimate of the sum of the quadratic deviations of factors, which will ensure optimal process control on a production scale.

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