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Osypchuk R. P.
PhD student, Department of Biology
Nizhyn Mykola Gogol State University
ruslan0399os@gmail.com
orcid.org/0009-0007-2193-0839

Kuchmenko O. B.
Doctor of Biological Sciences, Professor,
Head of the Biology Department of
Nizhyn Mykola Gogol State University
kuchmenko1978@gmail.com
orcid.org/0000-0002-3021-8583

THE CONTENT OF VITAMIN D2 IN BASIL SEEDS DURING SEED PRIMING WITH COMPOSITIONS OF METABOLICALLY ACTIVE COMPOUNDS

Basil (Ocimum basilicum L.) is found all over the world and is increasingly used in the food, pharmaceutical, and cosmetic industries. However, the nutritional and functional properties of its seeds have been little studied.

In the human body, vitamin D can be synthesized in the skin under the influence of ultraviolet radiation, followed by activation in the liver and kidneys to form the active form D3 - cholecalciferol. It can also be obtained from plant sources in the form of vitamin D2 (ergocalciferol). This vitamin performs a wide range of important functions, including optimizing mineralization processes in the body, inhibiting the proliferation of transformed cells, suppressing metastasis, inhibiting the activity of microorganisms, neutralizing reactive oxygen species, and participating in the maintenance of normal insulin secretion, among others.

The aim of this study is to determine the content of vitamin D2 in basil seeds and in the aqueous extract from these seeds using a modified determination method, as well as to evaluate the obtained results for statistical reliability.

As a result of the study, it was established that dry basil seeds have a significantly higher content of vitamin D2 compared to aqueous extracts from the same seeds. An effective agricultural approach to increasing the content of biologically active compounds in plant organisms is the pre-sowing treatment of seeds with various substances. As a result of the conducted studies, it was demonstrated that the most effective increase in vitamin D2 content in basil seeds was observed after pre-sowing treatment with combinations EPM (vitamin E + para-oxybenzoic acid + methionine) and EQ (vitamin E + ubiquinone-10). This may be related to the особенності of vitamin D2 biosynthesis in basil seeds and the influence of vitamin E, ubiquinone, para-oxybenzoic acid, and methionine on this process. By evaluating the correlation between relative frequencies and the frequencies of a normal distribution, it can be concluded that the method for determining vitamin D2 content in basil seeds using a modified colorimetric approach yields accurate results with low error. Determination of vitamin D2 content in basil seeds and in aqueous extracts from

basil seeds across all groups indicates the absence of background absorption and a small relative error.

These results may be useful in further studies of vitamin D2 content in plant organisms.

Keywords: basil seeds, vitamin D2, determination method, relative frequencies, normal distribution frequencies, background absorption

Introduction. Basil *(Ocimum basilicum* L.*)* is found worldwide and is increasingly used in the food, pharmaceutical, and cosmetic industries. However, the nutritional and functional properties of its seeds remain poorly studied. Basil seeds contain a high level of proteins (11.4–22.5 g/100 g), including all essential amino acids except tryptophan [1]. They also have a high content of linoleic acid (12–85.6 g/100 g) and minerals such as calcium, potassium, and magnesium.

Recently, basil seeds have been shown to contain phenolic compounds, including orientin, vicenin, and rosmarinic acid [2]. The importance of basil seed consumption for the prevention of type 2 diabetes and cardiovascular diseases has been demonstrated. In addition, basil extract has been proven to protect A549 cells from the effects mediated by infectious disease caused by *Klebsiella pneumoniae (Friedländer's bacillus)*, inhibiting cell death due to apoptosis [3].

It is also known that basil seed extract exhibits anti-inflammatory, antimicrobial, anti-ulcer, anticoagulant, and antidepressant properties [4].

To assess the state of the pro- and antioxidant balance in plants and their overall biochemical characteristics, the following indicators are currently used: the content of free radical oxidation products of lipids and proteins, bioflavonoids, SH groups, ascorbic acid, vitamins E, A, D, and C, the activity of catalase and superoxide dismutase, total antioxidant activity, etc. [1–4].

The content of these substances also depends on various agricultural practices applied during plant cultivation. One such practice is the pre-sowing treatment of seeds with compositions of metabolically active substances.

For this study, our attention was drawn to vitamin D_2. In humans, vitamin D is contained in two forms: D_3 (cholecalciferol), which is synthesized in the skin, and D_2 (ergocalciferol), which enters the body exclusively from the plant diet [10]. It performs a wide range of important functions, including optimizing mineralization processes in living organisms, inhibiting cancer cell proliferation, preventing metastasis, suppressing the activity of microorganisms, neutralizing reactive oxygen species, and maintaining normal insulin secretion, among others [5].

A commonly used method for determining vitamin D content is high-performance liquid chromatography; however, it is an expensive and hard-to-access procedure [11]. Therefore, we decided to improve a photocolorimetric method for determining vitamin D_2 in basil seeds [6], and to evaluate the obtained results for statistical reliability.

The aim of this study is to determine the content of vitamin D_2 in basil seeds and in the aqueous extract from these seeds using a modified determination method, as well as to evaluate the obtained results for statistical reliability.

Methods and Study Design. The study material consisted of basil seeds (*Ocimum basilicum* L.) and compositions of metabolically active substances: vitamin E (10^{-8} M), para-oxybenzoic acid (POBA) (0.001%), methionine (0.001%), ubiquinone-10 (10^{-4} M), and $MgSO_4$ (0.001%).

The study design included four variants:

- Control: seeds collected from plants pre-sowing treated with water.
- Group 1: seeds collected from plants pre-sowing treated with a composition of vitamin E (10^{-8} M) + POBA (0.001%) + methionine (0.001%) (EPM).

- Group 2: seeds collected from plants pre-sowing treated with a composition of vitamin E (10^{-8} M) + POBA (0.001%) + methionine (0.001%) + $MgSO_4$ (0.001%) (EPMMg).
- Group 3: seeds collected from plants pre-sowing treated with a composition of vitamin E (10^{-8} M) + ubiquinone-10 (10^{-4} M) (EQ)

Basil seeds were soaked for 6 hours in the respective solutions and then sown in open soil as four groups, spaced 3 meters apart, in mid-May 2024. Seeds from all four plant groups were harvested at the end of August 2024.

The seeds were dried in a well-ventilated room at 23–28 ℃ without direct exposure to sunlight.

Vitamin D_2 content was determined both in the seeds themselves and in an aqueous extract. The aqueous extracts were prepared by mixing water and dried plant material (basil seeds) in a 3:10 ratio, followed by extraction at 90 ℃ for 40 minutes.

Vitamin D_2 content was evaluated colorimetrically. The principle of the method is based on the qualitative reaction of vitamin D_2 with an aniline reagent, resulting in the formation of a red dye with an absorption spectrum at 420 nm [6].

Results and Discussion. The method for determining vitamin D_2 content was improved with modifications. The principle of the method involves extracting vitamin D_2 from plant material, followed by a qualitative reaction with an aniline reagent and measuring the color intensity using a photoelectrocolorimeter [6].

The aniline reagent is prepared by mixing aniline with concentrated hydrochloric acid in a 15:1 ratio in the presence of an excess of chloroform.

The reagents required for determining vitamin D_2 content are: vitamin D_2 of known concentration, ethanol, aniline reagent (aniline + HCl in a 15:1 ratio), and an 8% chloroform solution.

To begin, a calibration curve is constructed. For this, a standard solution of vitamin D_2 is prepared by dissolving 20 mg of pure vitamin D_2 in 100 cm^3 of 80% ethanol. To improve dissolution, the mixture is heated to 38 ℃.

Into wide test tubes, the following volumes of the standard vitamin D_2 solution (in cm^3) are added: 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 4.5, and 5.0. The volume of each solution is then brought up to 5 cm^3 with an 8% ethanol solution.

As a result, each test tube contains the following amounts of vitamin D_2, respectively: 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, and 1 mg.

To each test tube, 15 cm^3 of 8% chloroform solution is added, and the mixture is kept in the dark at 25 ℃ for 15 minutes. Then, 5 cm^3 of the aniline reagent is added to each test tube, and the mixture is left in the dark at 25 ℃ for 2 hours.

The resulting settled mixture is kept for 30 minutes at 40 ℃, after marking the volume, and 80% ethanol is added up to this mark.

To construct the calibration curve, the x-axis represents the vitamin D_2 content (mg), and the y-axis represents the optical density of the colored solutions. Measurements are performed using a photoelectric colorimeter at a wavelength of 420 nm.

Method. The plant extract is measured in milliliters in proportion to the mass of the dry material (taking into account the specifics of preparing the aqueous extract, as described in the Materials and Methods section). Depending on the expected vitamin D_2 content in the plant material, between 1 and 2 grams of dry material are used, corresponding to 4.66–23.3 milliliters of the aqueous extract.

Add 25 ml of 80% ethanol. Transfer the mixture into a 100 cm^3 volumetric flask and bring the volume up to the mark with ethanol, mixing thoroughly along the way. Let the mixture stand for 1 hour at 25 ℃.

Take 5 cm^3 of the mixture and transfer it into a test tube. Add 15 cm^3 of 8% chloroform solution and keep it in the dark at 25 ℃ for 15 minutes. Then add 5 cm^3 of the aniline reagent and allow the mixture to stand in the dark at 25 ℃ for 2 hours.

The resulting mixture is placed in a water bath for 30 minutes at 40 ℃ (after marking the volume and gradually adding 80% ethanol up to this mark). Afterward, the mixture is allowed to cool.

The control is prepared in the same way as the test mixture, but water is used instead of the aniline reagent. All measurements are performed on a photoelectric colorimeter at a wavelength of 420 nm, as this corresponds to the absorption spectrum of the red dye formed. This qualitative reaction between vitamin D_2 and the aniline reagent is known as the Furter reaction [6].

The vitamin D_2 content, expressed in mg per 100 g of plant material (mg%), is determined using the following formula:

$$x = \frac{C * V1 * V2 * 100}{m * V3 * V4}$$

where:

C - vitamin D_2 content in 5 cm^3 of extract, determined from the calibration curve;

V_1 - total volume of the ethanol solution with the extract;

V_2 - volume of the ethanol solution with the extract after incubation at 40 ℃;

V_3 - volume of the mixture taken for analysis;

V_4 - volume of the mixture taken for colorimetric measurement;

m - mass of dry material corresponding to the volume of extract taken.

Analyzing the results, it is evident that the level of vitamin D_2 in basil seeds is relatively low. However, there is a noticeable difference in its content between the experimental groups and the control group. It was found that the vitamin D_2 content in dry basil seeds from the first, second, and third experimental groups is significantly higher by 0.01, 0.006, and 0.01 mg/100 g, respectively, compared to the control group (Table 1).

Table 1

Vitamin D_2 content in basil seeds, mg/100 g (mg%)

Group	mg/100g (dry seeds)	mg/100g (aqueous extracts)
Control	0,0269 ± 0,0023	0,0016 ± 0,0002
Group 1	0,0367 ± 0,0027*	0,0015 ± 0,0002
Group 2	0,0329 ± 0,0018*	0,0019 ± 0,0003
Group 3	0,0367 ± 0,0027*	0,0016 ± 0,0001

*- probable differences ($p<0,05$) compared to control

When examining the vitamin D_2 content in the aqueous extracts, no significant differences were found either between the experimental groups or between the experimental groups and the control.

This may be related to the specific features of vitamin D biosynthesis in basil seeds and the influence of metabolically active substances on this process. The probable causes and mechanisms of how these substances affect vitamin D_2 biosynthesis in basil seeds will be discussed in more detail in subsequent publications.

From a statistical perspective, the results were analyzed by determining relative frequencies, frequencies for a normal distribution, and the correlation between relative frequencies and the frequencies of the normal distribution.

Relative frequencies are the proportion of the absolute frequency of a value within the total number of values in the dataset.

Frequencies of a normal distribution represent the frequency of random values that follow a normal (Gaussian) distribution. Essentially, this is a function that defines the ideal or expected value for each variable [7–9].

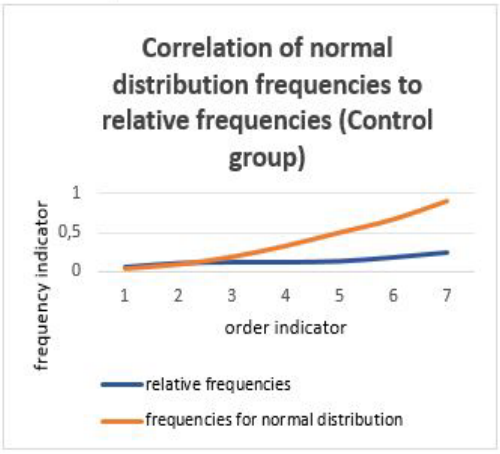
The correlation of these values helps to establish the relationship between the frequencies and determine whether the observed frequencies are close to the ideal predicted values [8-9].

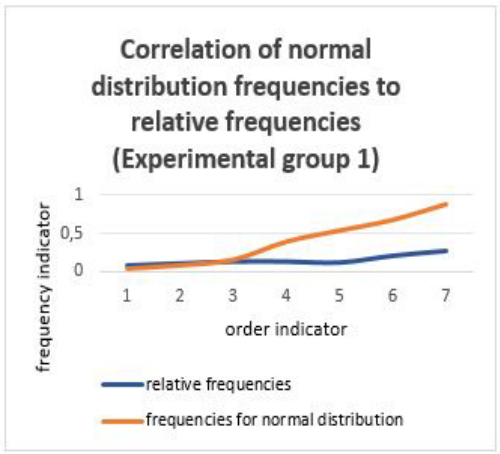
For each group, 80 replicates were performed to determine errors at each stage as accurately as possible.

When examining vitamin D_2 content in the control group, it was found that the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was 0.95 (where 0 = no correlation, 1 = maximum possible strong correlation [7]) (Fig. 1).

In the first experimental group (EPM), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was 0.89 (Fig. 2).

Fig. 1. Fig. 2.

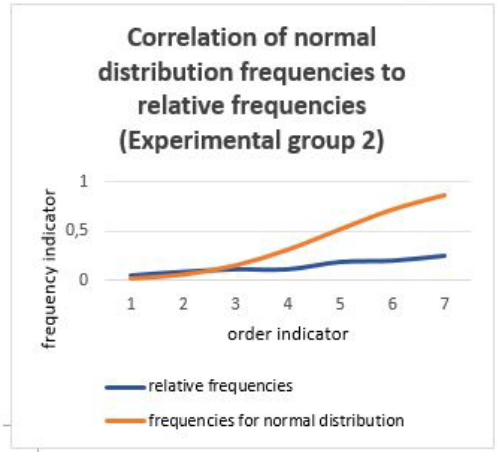


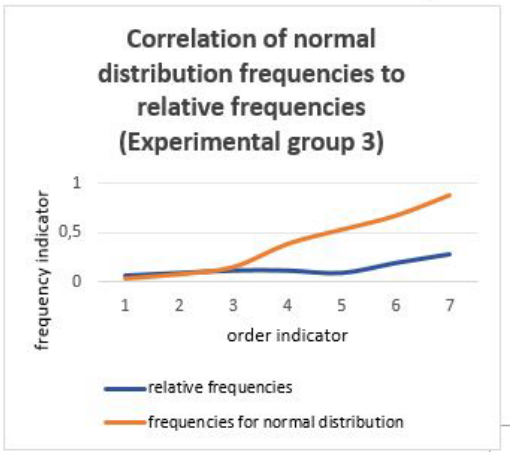


When examining vitamin D_2 content in the third experimental group (EPMMg), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was found to be 0.98 (Fig. 3).

For the fourth experimental group (EQ), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was 0.86 (Fig. 4).

Fig. 3. Fig. 4.





When examining vitamin D_2 content in the control group (aqueous extract of basil seeds), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was 0.93 (Fig. 5).

For the aqueous extract from the first experimental group (EPM), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was also 0.93 (Fig. 6).

Fig. 5.

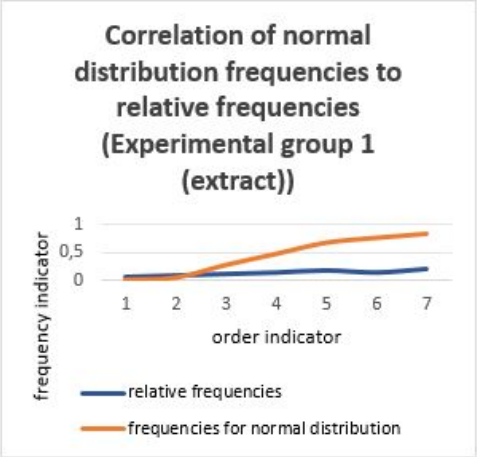
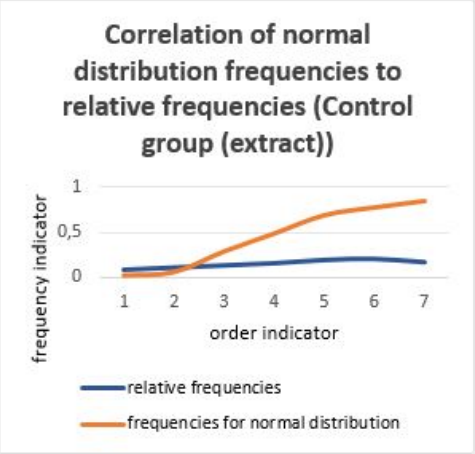


Fig. 6.



When examining vitamin D_2 content in the aqueous extract from the second experimental group (EPMMg), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was 0.88 (Fig. 7).

For the aqueous extract from the third experimental group (EQ), the correlation coefficient between the relative frequencies and the frequencies of a normal distribution was 0.90 (Fig. 8).

Fig. 7.

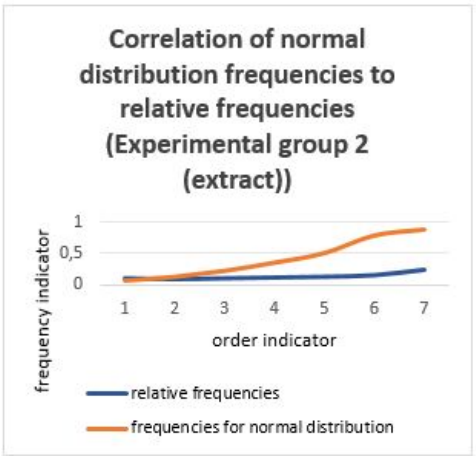
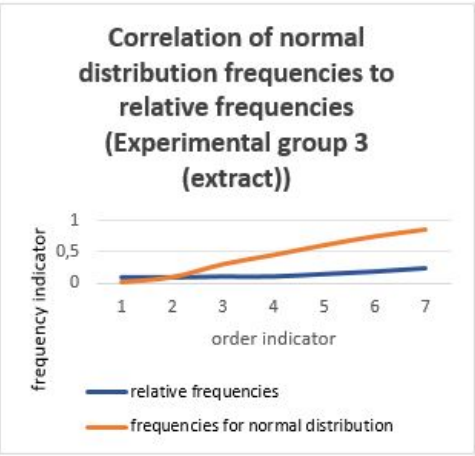


Fig. 8.



It can already be concluded that this method demonstrates high accuracy, as evidenced by the strong correlation between the relative frequencies and the frequencies of a normal distribution.

The specificity of the method is confirmed by the absence of background absorption and a negligible relative systematic error (Figs. 9–10).

Fig. 9

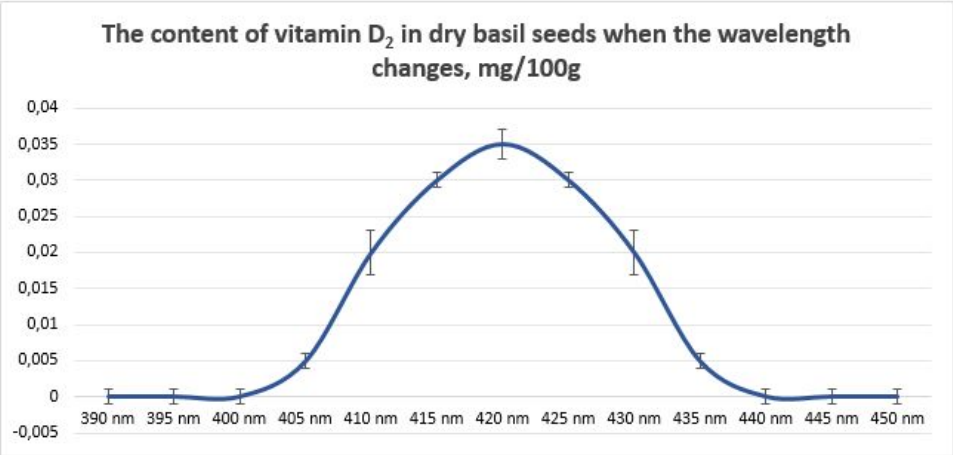
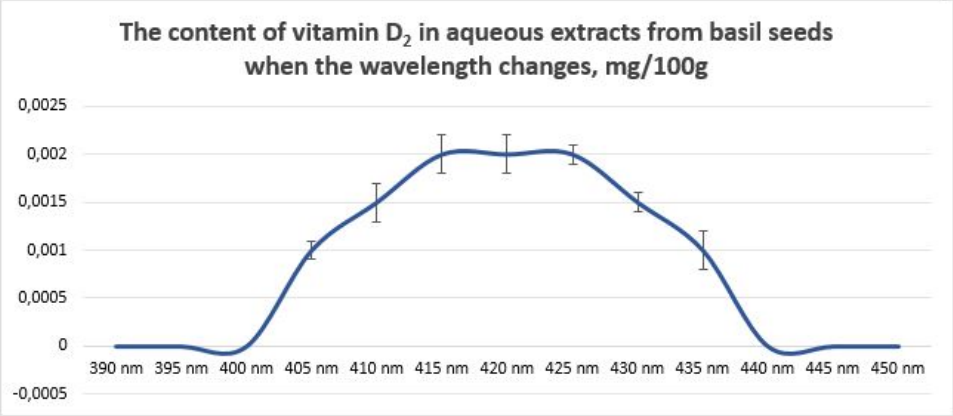


Fig. 10.



Conclusions. The study established that dry basil seeds contain significantly higher levels of vitamin D_2 compared to aqueous extracts from the same seeds. The most effective increase in vitamin D_2 content was observed in the EPM and EQ combinations (experimental groups 1 and 3). This may be related to the specific features of vitamin D_2 biosynthesis in basil seeds and the influence of vitamin E, ubiquinone, para-oxybenzoic acid, and methionine on this process.

By evaluating the correlation between the relative frequencies and the frequencies of a normal distribution, it can be concluded that the colorimetric method for determining vitamin D_2 content in basil seeds provides accurate results with a low margin of error.

When checking the vitamin D_2 content in basil seeds and in aqueous extracts from basil seeds across all groups, and by shifting the wavelength to both higher and lower values, it can be concluded that there is no background absorption and that the relative systematic error is negligible.

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Осипчук Р. П.
аспірант кафедри біології
Ніжинського державного університету імені Миколи Гоголя
ruslan0399os@gmail.com
orcid.org/0009-0007-2193-0839

Кучменко О. Б.
доктор біологічних наук, професор,
завідувач кафедри біології
Ніжинського державного університету імені Миколи Гоголя
kuchmenko1978@gmail.com
orcid.org/0000-0002-3021-8583

ВМІСТ ВІТАМІНУ D2 В НАСІННІ БАЗИЛІКА ЗА ПЕРЕДПОСІВНОЇ ОБРОБКИ КОМПОЗИЦІЯМИ МЕТАБОЛІЧНО АКТИВНИХ СПОЛУК

Базилік (Ocimum basilicum L.) зустрічається по всьому світу і все частіше його використовують в харчовій, фармацевтичній та косметичній промисловості. Однак харчові та функціональні властивості його насіння вивчені мало.

В організмі людини вітамін D здатен синтезуватись під дією ультрафіолетових променів в шкірі з подальшою активацією в печінці та нирках з утворення активної форми D_3 – холекальциферолу, а також надходити із рослинних джерел у вигляді вітаміну D_2 (ергокальциферолу). Цей вітамін виконує велику кількість важливих функцій, зокрема оптимізує процеси мінералізації в живому організмі, інгібує проліферацію трансформованих клітин, інгібує метастазування, інгібує життєдіяльність мікроорганізмів, нейтралізує активні форму кисню, бере участь в підтримці нормальної секреції інсуліну тощо.

Метою даного дослідження є визначення вмісту вітаміну D_2 в насінні базиліка та у водному екстракті з цього насіння за допомогою модифікованого методу визначення, а також оцінка отриманих результатів на статистичну достовірність.

В результаті дослідження було встановлено, що сухе насіння базиліка має значно вищий вміст вітаміну D_2, в порівнянні з водними екстрактами з цього ж насіння. Ефективним агропідходом до збільшення вмісту біологічно активних сполук в рослинних організмах є передпосівна обробка насіння різноманітними речовинами. В результаті проведених досліджень було продемонстровано, що найефективніше збільшення вмісту вітаміну D_2 в насінні базиліку спостерігалося за передпосівної обробки комбінаціями EPM (вітамін E + пара-оксибензойна кислота + метіонін) та EQ (вітамін E + убіхінон-10). Це може бути пов'язано з особливістю біосинтезу вітаміну D_2 в насінні базиліка та впливом на цей процес вітаміну E, убіхінону, параоксибензойної кислоти та метіоніну.

Оцінивши показники кореляції між відносними частотами та частотами нормального розподілу можна зробити висновок, що методика визначення вмісту вітаміну D_2 в насінні базиліка колориметричним методом з модифікаціями демонструє точні результати з малою похибкою. Визначаючи вміст вітаміну D_2 в насінні базиліка та у водних екстрактах із насіння базиліка в усіх групах можна говорити про відсутність фонового поглинання і незначну відносну похибку.

Ці результати можуть стати в нагоді в подальших дослідженнях вмісту вітаміну D_2 в рослинних організмах.

Ключові слова: насіння базиліка, вітамін D_2, методика визначення, відносні частоти, частоти нормального розподілу, фонове поглинання.

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