THE USE OF CHLORELLA SUSPENSION IN CROP PRODUCTION AND ITS EFFECT ON SEED GERMINATION OF AGRICULTURAL PLANTS

Lazzyat K. Orazzhanova*, Zhanar Zh. Nurtazina, Nuray B. Kiarstanova, Danara D. Beisenova

Shakarim University, 071410, Kazakhstan, Semey, st. Glinka, 20A

Abstract

The beet plant in the early period of development requires certain conditions for seed germination. Based on the biological characteristics of the crop, to obtain high yields of root crops, it is necessary to use non-toxic bio stimulants, such as chlorella microalgae. Chlorella microalgae, containing a complex of macro- and microelements in a biologically active form, promotes plant growth at all stages of vegetative development, and also provides protection against diseases and pests. This paper presents the results of the positive effect of treating the seeds of common beet Beta Vulgaris varieties Bresko F1 and EXP 3625 with a suspension of Chlorella vulgaris. The best bio stimulating and economic effect was shown by a chlorella suspension with a concentration of 14 million cells/ ml.

Keywords: *Chlorella vulgaris, Beta Vulgaris, germination, germination energy*

1. INTRODUCTION

Chlorella is a microalgae discovered in the late 19th century by the Dutch scientist Martinus Beijerinck. Over the years of research, chlorella continues to arouse interest and is studied by scientists in various fields of science and technology, including the medical, pharmaceutical, biotechnology industry, agriculture and plant growing [1]. The high content of biologically active substances (BAS) - proteins, carbohydrates, lipids, vitamins and minerals, as well as a combination of immunostimulating bioactive compounds such as antioxidants, polyunsaturated fatty acids, enzymes, phytohormones, chlorella suspension helps improve metabolic processes, increases the body's resistance to pathogenic flora, and enhances the energy potential of cells and tissues. Due to their unique biological properties, microalgae have found wide application in plant growing as a valuable biofertilizer - a source of functional ingredients. Also, the introduction of chlorella suspension into the soil layer stimulates beneficial microbiota and helps to structure the soil to increase its fertility [2]. The combination of the above properties of chlorella promotes the full development of plants at all stages of the life process and increases crop yields. A number of studies indicate that the treatment of beet seeds with a suspension of Chlorella vulgaris promotes faster germination, increases germination energy and increases overall germination. One of the promising areas of application of chlorella suspension is pre-sowing seed treatment, which allows activating the physiological and biochemical processes responsible for germination. It has been established that chlorella components, including phytohormones and antioxidants, promote the activation of enzymatic activity, enhance the respiratory activity of the embryo and ensure faster emergence of seedlings. This is especially important for crops with a sensitive initial growth phase, such as beets, where uniform and rapid germination determines future yields [3-5]. Due to its environmental friendliness and high efficiency, Chlorella vulgaris is becoming increasingly popular as a sustainable alternative to synthetic agrochemicals. The aim of this study is to use Chlorella suspension to improve the germination of *Beta Vulgaris* seeds.

2. MATERIALS AND METHODS

2.1. Materials

The objects of the study were a suspension of the microalgae *Chlorella vulgaris* and seeds of common beet *Beta Vulgaris* of two varieties Bresko F1 and EXP 3625. The microalgae seed material was tested for purity and viability before the experiment. The seeds of common beet (*Beta vulgaris*) of the Bresko F1 and EXP 3625 varieties were pre-calibrated and tested for mechanical damage and diseases. All

materials used (reagents, water, glassware) met the requirements of analytical purity and sterility. Object and cover glasses, a Goryaev chamber, and a laboratory light microscope were used to conduct microscopic analyses.

2.2. Methods

Microalgae were cultivated using the commercial strain *Chlorella vulgaris* IFR No. C-111 on Bogdanov's nutrient medium with the following composition: NH_4NO_3 – 0.1 g/dm^3, $NH_4H_2PO_4$ – 0.01 g/dm^3, 1% solution of $FeCl_3 \cdot 6H_2O$ – 0.15-0.17cm^3, 0.01% solution of $Co(NO_3)_2$ – 0.5-0.6 cm^3, 0.01% solution of $CuSO_4$ - 0.5-0.7 cm^3, 12% solution of K_2SO_4 – 0.33-0.39 cm^3, $MgSO_4 \cdot 7H_2O$ – 0.2 g/dm^3.

Nitrogen in the form of nitrates NH_4NO_3 is necessary for the synthesis of amino acids, proteins and nucleic acids. Phosphorus, coming from $NH_4H_2PO_4$ is involved in energy metabolism and stabilization of the pH of the environment. Iron, which is part of $FeCl_3 \times 6H_2O$, is necessary for the functioning of photosystems and respiratory enzymes. Magnesium from $MgSO_4 \times 7H_2O$ is part of chlorophyll and activates enzymatic processes [6-7].

The *Chlorella vulgaris* microalgae cultivation procedure was carried out under controlled laboratory conditions, in a specially prepared light-tight growbox, providing a stable environment for the growth of microalgae. The temperature in the growbox was maintained at 28±2°C, which corresponds to the optimal conditions for the active growth of chlorella. Lighting was provided by DNaT lamps with a full spectral characteristic simulating daylight. The luminous flux was 3000 lux, which ensured the required intensity of photosynthetic processes. The acidity of the medium was maintained in the pH range of 7–9, which is most favorable for the metabolic activity and biosynthesis of the cellular mass of microalgae. The photoperiod was set at a ratio of 16 hours of light exposure and 8 hours of the dark phase, which corresponded to the natural day and night regime under vegetative growth conditions. The culture liquid was placed in 250 ml glass conical flasks, each filled with 200 ml of the mixture in a volume ratio of 40 ml of the inoculation suspension and 160 ml of the Bogdanov's nutrient medium. To prevent sedimentation and ensure uniform distribution of cells throughout the volume of the culture liquid, the flasks were subjected to constant aeration. Air was supplied through aerators at a rate of 3 dm^3/h. Carbon dioxide (CO_2) at a concentration of 0.03% contributed to the activation of photosynthesis and accelerated biomass growth. All cultivations were carried out with triplicate biological replication and in three analytical measurements to ensure statistical reliability of the results (Fig. 1). The flasks were placed at an equal distance from each other to exclude light and temperature gradients. Parameters were monitored daily, including measurements of temperature, pH, illumination level and visual assessment of the culture condition.

Fig. 1. Cultivation of *Chlorella vulgaris* and germination of *Beta Vulgaris* seeds in a growbox

Biomass growth was determined by optical and microscopic methods. The optical density of the suspension was measured daily for 5 days using a Specord 210 plus UV visible spectrophotometer (Germany) at a wavelength of 683 nm [8-11].

Microscopic examination of the chlorella suspension was performed by daily counting of the total number of cells in a Goryaev chamber at a magnification of ×400 using a LOMO light binocular microscope (Russia) (Fig. 2). The images of cells obtained under microscope magnification were used to visually confirm the viability of the culture, the homogeneity of the cell mass and the absence of contamination. To ensure the reliability of the data, the cell count was performed in three technical replicates with subsequent calculation of the mean value and standard deviation. Before use, the suspension was thoroughly mixed for 5 minutes to ensure uniform distribution of the cells. The number of microalgae cells in 1 ml of the studied suspension was determined by the formula:

$$X = m \cdot 10^4,$$

where m - the total number of microalgae cells in the large grid squares taken into account, 10^4 - the conversion factor for cubic millimeters to cubic centimeters.

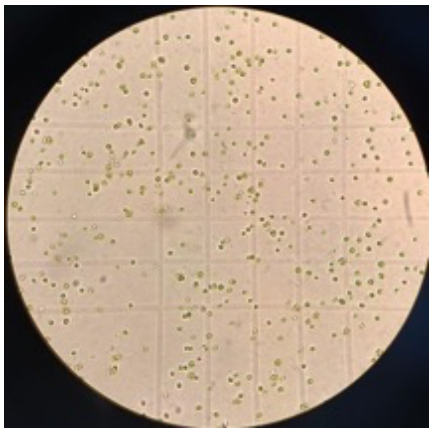


Fig. 2. Microscopic image of Chlorella vulgaris microalgae cells in Goryaev's chamber

Beet seeds were germinated on corrugated paper in sterile transparent plastic germinators with the following dimensions: length 18 cm, width 10 cm, height 5 cm. Corrugated paper 28-30 cm long and 10 cm wide had 10 folds with a tooth height of 15-20 mm. Corrugated paper was dipped in chlorella suspension, excess was allowed to drain off and placed in a germinator. The total volume of chlorella for wetting the paper was 15 ml. 5 seeds were placed in each fold, the number of seeds was 50 pieces in one germinator. The prepared germinators with seeds were placed in a light-proof growbox and germinated at a temperature of 20-30^{0}C, the lids of the germinators were opened twice a day and aired for 30 minutes. The moisture content of the bed was checked daily and, if necessary, it was moistened with bidistilled water at room temperature, avoiding over-moistening. The evaluation and recording of germinated seeds was carried out by determining the germination energy (E, %) and the length of the sprouts (cm) on the 5th day, laboratory germination (B, %) and the length of the sprouts (cm) - on the 10th day according to formula 1 [12-13].

$$B(E) = \frac{N_g}{N_t} \cdot 100\% \quad (1)$$

where N_g is the number of germinated seeds, N_t is the total number of seeds.

In a laboratory experiment, seed treatments with various concentrations of a suspension of chlorella microalgae with a cell density of: 1. 32 million cells/ml, 2. 14 million cells/ml, 3. 7 million cells/ml were studied. Bidistilled water was used as a control. The experiment was repeated three times.

3. RESULTS AND DISCUSSION

During the entire period, a gradual increase in the optical density of the culture was observed, indicating active growth of the microalga. The increase in the number of cells also confirms the adaptation of the strain to the nutrient medium used and optimal cultivation conditions. The results of the 4-day cultivation cycle of Chlorella vulgaris are presented in Table 1.

Day	Optical density	Number of cells, million cells/ml
1	0.9829	1.3
2	0.9871	1.5
3	1.0933	1.7
4	1.0974	1.8

Table 1. Dynamics of Chlorella growth in Bogdanov medium.

By the fourth day of cultivation, the optical density of the *Chlorella vulgaris* suspension reached 1.0974, indicating a pronounced and intensive growth of biomass in the studied system. Such an indicator indicates active photosynthetic functioning of cells and efficient use of the nutrient medium components. Along with this, an increase in the total number of cells in the suspension was recorded, reaching 1.8 million cells per milliliter, which also confirms the high degree of viability of the microalgae under the given cultivation conditions. These data demonstrate successful adaptation of the culture to the experimental environment and favorable dynamics of cell mass accumulation [14].

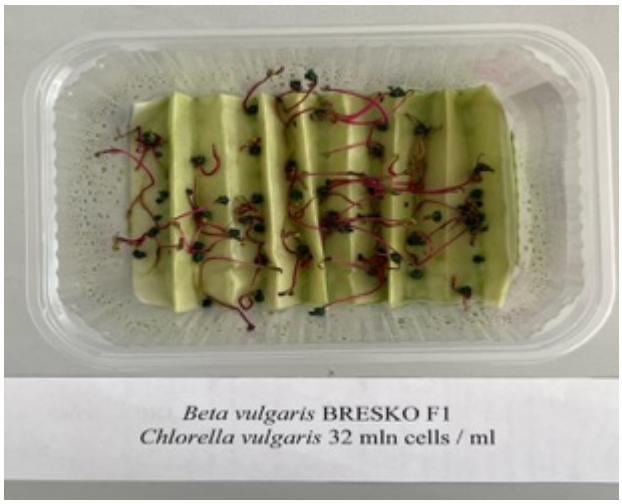
Subsequently, the resulting suspension was used to treat common beet seeds (*Beta vulgaris*) in order to study the effect of different concentrations of cell density on their germination rates. The results of laboratory experiments conducted using chlorella suspension as a biostimulant are presented in Table 2 and also visualized in Fig. 3. They reflect the effect of treatment on such parameters as germination energy, laboratory germination and length of seedlings at various stages of development.

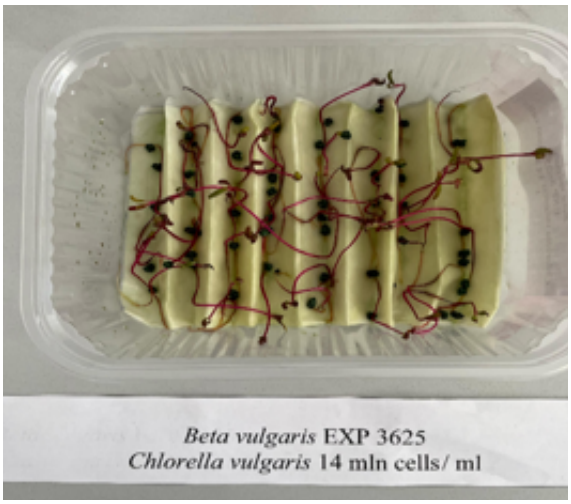
The analysis of the obtained data allows us to draw a conclusion about the dependence of the physiological characteristics of seeds on the concentration of the chlorella suspension [15]. The most pronounced stimulating effect was observed when using medium and high concentrations of cell density, while at a low concentration the increase in indicators was minimal or absent. These results confirm the feasibility of using *Chlorella vulgaris* as an effective biological stimulator in the early stages of ontogenesis of agricultural crops.

Experimental options	**Seed viability indicators, %**		**Length of sprouts, cm**	
	Germination energy	Laboratory germination	On the 5th day	On the 10th day
	Bresko F1			
Control	36%	60%	2.12	2.86
Moistening seeds with chlorella suspension (32 million cells/ml)	94%	98%	2.5	4.15
Moistening seeds with chlorella suspension (14 million cells/ml)	86%	94%	2.5	3.8
Moistening seeds with chlorella suspension (7 million cells/ml)	24%	48%	2.125	3.8

		EXP 3625		
Control	84%	92%	2.9	3.7
Moistening seeds with chlorella suspension (32 million cells/ml)	76%	84%	3.24	5.2
Moistening seeds with chlorella suspension (14 million cells/ml)	86%	86%	4.15	6.95
Moistening seeds with chlorella suspension (7 million cells/ml)	28%	34%	2.2	3.794

Table 2. Effect of treatment with a suspension of *Chlorella vulgaris* microalgae on the viability of Beta Vulgaris beet seeds, Bresko F1 and EXP 3625 varieties





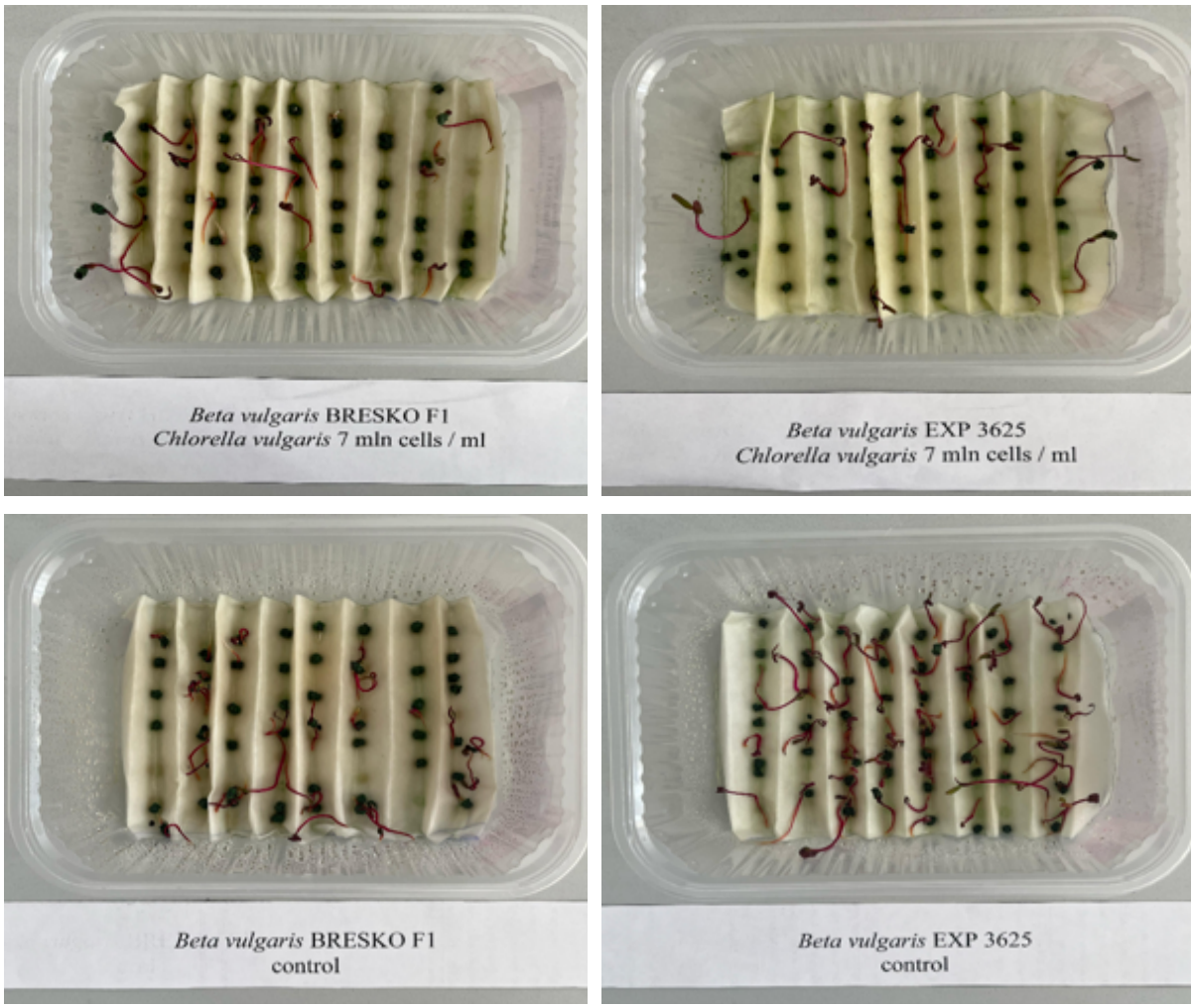


Fig. 3. Results of germination of seeds of common beet *Beta Vulgaris*

As can be seen from Table 2, when seeds are moistened with a chlorella suspension with cell concentrations of 32 million cells/ml and 14 million cells/ml, the sowing qualities of seeds of both varieties improve. At the same time, the Bresko F1 variety has higher seed viability indicators. For example, for seeds of the Bresko F1 variety, the indicators increase as follows: germination energy - by 58%, laboratory germination by 38%, sprout length by 15% (on day 5) and by 31% (on day 10). For seeds of the EXP 3625 variety, the indicators increase as follows: germination energy - by 50%, laboratory germination by 34%, sprout length by 15% (on day 5) and by 25% (on day 10).

4. CONCLUSIONS

Thus, seed treatment with moistening with chlorella suspension with cell concentrations of 32 and 14 million cells/ml is an effective method of biostimulation of seed germination in laboratory conditions. However, in agricultural production conditions, in order to reduce the cost of chlorella suspension, it is recommended to use chlorella suspension with a concentration of 14 million cells/ml. The obtained data can be used in the development of agricultural technologies aimed at increasing crop yields and improving the initial stages of growth of agricultural crops.

ACKNOWLEDGMENTS

This research has been/was/is funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP AP23488216).

REFERENCES

1. Khan, M, et., 2018, "The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products", *Microbial Cell Factories*, vol.17, p.36.
2. Bruna, SV, Juliana, BM, Michele, GM, Jorge, AVC 2016, "Microalgae as a new source of bioactive compounds in food supplements", *Current Opinion in Food Science*, vol. 7, pp.73-77.
3. Puglisi, I, Barone, V, Fragalà, F, Stevanato, P, Baglieri, A & Vitale, A 2020, "Effect of microalgal extracts from *Chlorella vulgaris* and *Scenedesmus quadricauda* on germination of *Beta vulgaris* seeds", *Plants*, vol. 9, no. 6, article 646
4. Lin, D, et, 2024, "Effects of the supernatant of Chlorella vulgaris cultivated under different culture modes on lettuce (Lactuca sativa L.) growth", *Frontiers in Nutrition*, vol.11, article 1437374.
5. Machneva, N 2021, "Biotechnological peculiarities of microalgae chlorella production and use in poultry", E3S Web of Conferences 285, article 03002.
6. Golodova, IV, Vassilyev, NV, Soldatova, VA, Reibandt, AI 2020, *Planktonic strain of unicellular green algae Chlorella vulgaris SKO specialized for biomass production*, KZ Patent 35004
7. Bogdanov, NI 2018, *Thermophilic plankton strain Chlorella Sorokiniana product food biomass*, RU Patent 2680704.
8. Politaeva, NA, Smyatskaya, YA, Kuznetsova, TA, et al. 2017, "Kultivirovanie i ispol'zovanie mikrovodoroslei Chlorella i vysshikh vodnykh rastenii ryaska Lemna", Saint Petersburg State Polytechnic University of Peter the Great, Saint Petersburg, Nauka Publishing House, p. 87
9. Wong, YK, Ho, H, Ho, KC, Leung, HM & Yung, K 2017, "Growth medium screening for Chlorella vulgaris growth and lipid production", *Journal of Aquaculture & Marine Biology*, vol. 6, no. 1, article 00143.
10. Daliry, S, Hallajisani, A, Mohammadi Roshandel, J, Nouri, H & Golzary, A 2017, "Investigation of optimal condition for Chlorella vulgaris microalgae growth", *Global Journal of Environmental Science and Management*, vol. 3, no. 2, pp. 217–230
11. Deniz, I 2020, "Determination of growth conditions for *Chlorella vulgaris*", *Marine Science and Technology Bulletin*, vol. 9, no. 2, pp. 114-117
12. Pawłat, J, Starek-Wójcicka, A, Kopacki, M, Terebun, P, Kwiatkowski, M, Sujak, A, Pascuzzi, S, Santoro, F & Andrejko, D 2022, "Germination energy, germination capacity and microflora of Allium cepa L. seeds after RF plasma conditioning", *Energies*, vol. 15, article 7687
13. Pradheeban, L, Nissanka, S & Suriyagoda, L 2013, "Clustering of rice (Oryza sativa L.) varieties cultivated in Jaffna District of Sri Lanka based on salt tolerance during germination and seedling stages", *Tropical Agricultural Research*, vol. 25, pp. 146–162
14. Daliry, S, Hallajisani, A, Mohammadi Roshandel, J, Nouri, H & Golzary, A 2017, "Investigation of optimal condition for Chlorella vulgaris microalgae growth", *Global Journal of Environmental Science and Management*, vol. 3, no. 2, pp. 217–230
15. Puglisi, I, Barone, V, Fragalà, F, Stevanato, P, Baglieri, A & Vitale, A 2020, "Effect of microalgal extracts from Chlorella vulgaris and Scenedesmus quadricauda on germination of Beta vulgaris seeds", *Plants*, vol. 9, no. 6, article 646