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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

INVESTIGASION OF THE ANTIOXIDANT SYSTEM OF *DUNALIELLA SALINA IPPAS D-294* CELLS AT LOW TEMPERATURE STRESS CONDITIONS

Specialty: 2406.01 – Biophysics

Field of science: Biology

Applicant: **Aynura Rafik Jalilova**

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The work was performed at the "Bioengineering" Laboratory of the Excellence Center for Research, Development and Innovations of Baku State University.

Scientific supervisor: Doctor of Physics-Mathematics Sciences, Professor
Rovshan Ibrahimkhalil Khalilov

PhD in Biological Sciences, Associate Professor
Gafar Islam Ali-zadeh

Official opponents: Doctor of Biological Sciences, Professor
Tokay Maharram Huseynov

Doctor of Physics-Mathematical Sciences, Professor
Ahmed Mahammad Hajiyev

PhD in Biological Sciences, Associate Professor
Gulnar Hajibey Sultanova

Dissertation council of FD 2.31 of the Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Baku State University.

Chairman of the Dissertation council: Doctor of Biological Sciences, Professor
Ralfrid Ahadovich Hasanov

Scientific secretary of the Dissertation council: PhD in Biological Sciences, Associate Professor
Samira Jafar Salayeva

Chairman of the scientific seminar: Doctor of Physics-Mathematics Sciences, Professor
Eldar Ali Masimov

Azərbaycan Respublikası Təhsil Nazirliyi
BAKI DÖVLƏT UNİVERSİTETİ
Ministry of Education of the Republic of Azerbaijan
Baku State University

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SCIENTIFIC SECRETARY
İmzanı təsdiq edirəm:
Y.N.ƏLİYEVA
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GENERAL DESCRIPTION OF WORK

Relevance of the topic. Modern science pays great attention to the problem of plant resistance to low positive temperatures[1].

Although several theories have been put forward regarding the damaging effect of low temperatures on plants, plants that are resistant to frost are the object of them. The main cause of damage to heat-loving plants under the action of low lethal temperatures is the formation of reactive oxygen species (ROS) [2,3].

As a result of studies carried out in recent years, it has been established that the generation of reactive oxygen species and the development of oxidative stress are one of the responses of plants to the damaging effects of various abiotic factors. Antioxidant activity increases as a result of increased ROS generation. Antioxidant enzymes play an important role in protecting metabolic processes from damaging effects. But they quickly lose activity when the redox status inside the cell changes[4,5]. Under the action of stress factors, plant cells accumulate low molecular weight organic antioxidants - proline, polyamines, anthocyanins, carotenoids, flavonoids, soluble phenols, etc.) [6]. They are protective compounds under oxidative stress.

Under the influence of various adverse environmental factors, in order to find out the species-specific features of the functioning of the antioxidant defense system, an extended introduction of model plants is needed. This is one of the promising approaches to this issue. The

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[1] Трунова, Т.И. Растение и низкотемпературный стресс: 64-е Тимиряз. чт. / Т.И. Трунова, - Москва: Наука, - 2007. - 54

[2] Балнокин, Ю.В. Растения в условиях стресса / Ю.В. Балнокин // под ред. Ермакова. М: Издтельский центр «Академия», - 2005, - 640 с.

[3] Владимиров, Ю.А. Свободные радикалы в биологических системах // Соросовский образовательный журнал. – Москва: - 2000. 6 (12), - с. 13-19.

[4] Halliwell, B. Reactive Species and Antioxidants. Redox Biology Is a Fundamental Theme of Aerobic Life // Plant Physiology, - 2006. v. 141, p. 312-322

[5] Ignatenko, A.A. Effect of Salicylic Acid on Antioxidant Enzymes and Cold Tolerance of Cucumber Plants. / A.A. Ignatenko, V.V. Talanova, N.S. Repkina [et al.] // Russ J Plant Physiol, - 2021. 68, - p. 491–498

[6] Lamers, P.P. Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. / P.P. Lamers, C.C.W. Van De Laak, P.S. Kaasenbrood [et al.] // Biotechnol. Bioeng. – 2010. v. 106, - p. 638–648

biological object of such studies is the green microalgae *Dunaliella salina IPPAS D-294*.

Tolerance of green microalgae *Dunaliella salina IPPAS D-294* to various ranges of UV-B radiation directly depends on the conditions of existence: temperature, salinity, mineral nutrition. Currently, there is no sufficient knowledge about the mechanisms of plant tolerance to damaging factors under the action of UV-B radiation on plants.

The study of the mechanisms of resistance of *Dunaliella salina IPPAS D-294* cells to UV-B irradiation, the action of low temperatures, salinity, and their combination provides new fundamental knowledge about plant adaptation to adverse conditions.

Purpose and objectives of the study. The aim of this study was to study the endogenous activity of catalase, the process of lipid peroxidation and resistance to the subsequent action of various acute doses of UV-B radiation under optimal conditions and under conditions of low temperature stress and high salinity in cells *Dunaliella salina IPPAS D-294* in the presence of various concentrations of exogenous synthetic antioxidants.

To achieve this goal, the following tasks were set:

1. Evaluation of bioproductivity and functional activity of cell population *Dunaliella salina IPPAS D-294* grown under optimal conditions and under conditions of low temperature stress and high salinity;
2. Evaluation of bioproductivity and functional activity of cell population *Dunaliella salina IPPAS D-294*, modified with various concentrations of synthetic antioxidants, grown under optimal conditions and under conditions of low temperature stress and high salinity;
3. Study of endogenous catalase activity, levels of lipid peroxidation under optimal conditions and under conditions of low temperature stress at normal and high salinity in the presence of synthetic antioxidants;
4. Determination of indicators of cell resistance *Dunaliella salina IPPAS D-294* to the subsequent action of UV-B radiation, under optimal conditions and under conditions of low temperature stress and high salinity.

Scientific novelty. It has been established that functional stability manifests itself due to increased intracellular biosynthesis of the amount of carotenoids associated with high salinity of the mineral environment and low-temperature stress, endogenous catalase activity, lipid peroxidation levels and exogenous synthetic antioxidants that reduce the intensity of oxidative stress. It has been shown that *Dunaliella salina İPPAS D-294* cells grown under conditions of low temperature stress and high salinity, compared with control cells, exhibit increased resistance to subsequent exposure to various acute doses of UV-B radiation.

Theoretical and practical significance of the research. The results obtained deepen and expand knowledge about the mechanisms of plant adaptation to low-temperature stress and can be used to increase their stress resistance.

The revealed patterns of the growth-stimulating action of synthetic antioxidants (2,6 di-*tert*-butyl cresol (ionol) and 2,6 di-*tert*-butyl phenol) make them reliable and affordable growth activators and make it possible to effectively use them to regulate plant bioproductivity under extreme conditions. The results of the studies can be used to develop the basic principles for assessing the functional stability and the possibility of using cells of green microalgae *Dunaliella salina İPPAS D-294* as promising test objects for genetic monitoring, in assessing the biological consequences of abiotic environmental factors.

The dissertation materials can be used in lecture courses for students of biological, ecological and agricultural specialties.

Defense provisions.

1. The functional stability of the cells of green microalgae *Dunaliella salina İPPAS D-294* is manifested due to increased intracellular biosynthesis of total carotenoids associated with high salinity of the mineral environment and low-temperature stress, endogenous catalase activity, the degree of lipid peroxidation and exogenous synthetic antioxidants that reduce the intensity of oxidative stress.
2. Cells of green microalgae *Dunaliella salina İPPAS D-294*, grown under conditions of low temperature stress and high salinity, in comparison with control cells, show increased resistance to the subsequent action of various acute doses of UV-B radiation.

Approbation and implementation. The research results were

reported and discussed at the Congress of the Russian Photobiological Society (village Shepsi, 2014), V Congress of the Russian Biophysical Society (Rostov-on-Don, 2015), VI International Scientific Conference "Innovative Problems of Modern Biology" (Baku, 2016), scientific conference dedicated to the 90th anniversary of academician J. Aliyev (Baku, 2018), at a scientific conference dedicated to the 90th anniversary of the corresponding member of ANAS, Honored Scientist, Professor D. Hajiyev (Baku, 2019).

Publications. 31 scientific publications were published on the research topic (26 articles, 5 materials conference), of which 4 articles are included in the list of foreign scientific journals recommended by the Higher Attestation Commission (HAC).

The structure and scope of the dissertation. The dissertation is presented on 168 pages of computer text, consists of: introduction (11455), five chapter (I – 55418, II – 18655, III – 46716, IV – 17888, V – 20688), literature review, object and methods of research, results and their discussion, conclusion (41165), conclusions (2408), practical recommendations (1013), list of references, list of abbreviations; illustrated with 3 tables and 38 figures. The literature index contains 200 sources. The total volume of the dissertation is 275143 characters

BASIC CONTENT OF WORK

I CHAPTER. LITERATURE REVIEW

This chapter extensively discusses the damaging effect of low temperatures on plants and secondary oxidative stress, the role of the antioxidant system in the formation of plant resistance to low temperatures, the mutual functioning between the components of the antioxidant system, cold tolerance and cross-tolerance, as well as studies presented in this area around the world. with reference to literary sources.

II CHAPTER. OBJECT AND SUBJECT OF STUDY

In accordance with the purpose and objectives of the work, *Dunaliella salina IPPAS D-294*, isolated from the salt lakes of Apsheron and

introduced into culture, served as the object of study[7]. The culture of microalgae was grown on the installation, type "UVKV" (installation for growing cultures of microalgae). Cells are grown in the facility in the intensive-accumulation mode. Cell suspension in photoreactors was illuminated with white light (16 W/m^2) for 24 hours and continuously purged with a mixture (air + 1.0% CO_2) with a temperature of 27°C for optimal growing conditions. The cell suspension in photoreactors was purged for 24 hours with a mixture at a temperature of 27°C for control and 5°C for test suspensions (low temperature stress)[8]. Various concentrations of sodium chloride (1.5 M and 3.0 M NaCl) were added to the mineral cell growth medium. For each liter of nutrient medium, 1 ml of microelement solution was added, and the pH of the nutrient medium was adjusted with 0,1 N NaOH solution to 7,2-7,4. To irradiate the object with UV-B radiation, a high-pressure mercury lamp SVD-120A was used. The bioproductivity of the microculture was determined periodically by counting the number of cells under a microscope on the Goryaev chamber and nephelometric - on a photoelectrocolorimeter by measuring the optical density of the cell suspension.

Synthetic antioxidants 2,6 di-*tert*-butyl cresol (ionol) and its analog 2,6 di-*tert*-butyl phenol were used in the work at concentrations of 25–500 mkM.

The amount of total pigments in cell extracts (100% acetone) was determined on a spectrophotometer and calculated based on the Wettstein coefficients[9]. The rate of oxygen release by cells was measured using a polarographic setup using a platinum Clark electrode, illuminating the suspension (optical density *OD* = 0,8) in a thermostated

[7] Ализаде, Г.И., Магеррамова, Х.Х., Абдуллаев, Х.Д. Биопродуктивность водорослей в фотореакторах с усиленным перемешиванием // Экология, Философия, культура. Сб.науч. ст., - Баку: - 2004. - с.67-75

[8] Alizadeh, G.I. The antioxidative activity of *Dunaliella* cells under low temperature stress / G.I. Alizadeh, A.R. Jalilova, Kh.Kh. Maharramova1 [et al.] // International Journal of Biopharmaceutical and Nanomedical Sciences, IJBNS, - 2013. 2 (1), - p. 74-78

[9] Гавриленко, В.Ф., Ладыгина, М.Е., Хандобина, Л.М. Большой практикум по физиологии растений: [в 1 томе] / В.Ф. Гавриленко, М.Е. Ладыгина, Л.М. Хандобина – Москва: Высшая школа, - 1975. - 92 с.

volume with white light of saturating intensity (100 W/m^2) [10].

Determination of the degree of lipid peroxidation (LPO) was carried out according to the method for determining the content of MDA in cells - a method based on the reaction with thiobarbituric acid[11]. Catalase activity was measured by the gasometric method, which is based on determining the volume after adding hydrogen peroxide to an aqueous plant extract containing catalase.

The obtained data were processed using Microsoft Excel 2016 and processed statistically according to Lakin[12].

III CHAPTER. GROWTH, BIOSYNTHESIS OF PIGMENTS AND FUNCTIONAL ACTIVITY OF GREEN MICROALGAE *DUNALIELLA SALINA IPPAS D-294* CELLS

3.1. Growth, biosynthesis of pigments and functional activity of *Dunaliella salina IPPAS D-294* green microalgae cells under optimal and low-temperature cultivation (1,5 M NaCl)

The optical density of control cell suspensions under optimal conditions (25^{0}C) increases by 3,5-4 times. Under low-temperature stress (5^{0}C), the growth dynamics of the experimental suspension is and low-temperature stress, the ratio of chlorophylls *a* and *b* does not suppressed and the difference is 20-25%. Under conditions of optimal change (2:1), and the ratio of chlorophylls / carotenoids, which shows the photosynthetic activity of cells, decreases due to the high total carotenoids from 5,5 to 3,87 (Table 3.1.1.).

Low-temperature stress increases the biosynthesis of total carotenoids by *Dunaliella salina IPPAS D-294* cells, which affects the functional activity of algae. Algae grown under low-temperature stress conditions reduce functional activity by 35% compared to control.

This decrease in functional activity leads to suppression of cell

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[10] Масюк, Н.П. Морфология, систематика, экология, географическое распространение рода Dunaliella Teod. и перспективы его практического использования [в 1 томе] / Н.П. Масюк, - Киев: - 1973,- 244 с.

[11] Плешков, Б.П. Практикум по биохимии растений / Б.П. Плешков. - Москва: - 1976. - 255с.

[12] Лакин, Г.Ф. Биометрия [в 1 томе] / Г.Ф. Лакин // Высшая школа, - Москва: - 1990. - 293 с.

bioproductivity indicators[13,14].

Table 3.1.1.

The content of pigments in *Dunaliella salina IPPAS D-294* cells grown in the modesoptimal and low temperature cultivation

Mode cultivation	Chlorophyll *a* mg/l	Chlorophyll *b* mg/l	Sum Carotenoids mg/l	The ratio of chlorophylls/ carotenoids
K	3,27 ± 0,05	1,68 ± 0,05	0,9 ± 0,01	5,5
O	3,46 ± 0,05	1,73 ± 0,05	1,34 ± 0,01	3,87

Note: optical density *OD=0,8:* Temperature 27^0C, light intensity 16 W/m^2; K-blowing in photoreactors of an air mixture with a temperature of 25^0C; O-blowing in photoreactors of an air mixture with a temperature of 5^0C (low-temperature stress)

3.2. Growth, pigment composition and functional activity of *Dunaliella salina IPPAS D-294* green microalgae cells modified with exogenous antioxidants under optimal and low-temperature cultivation (1,5M NaCl)

In the optimal cultivation mode (25^0C) ionol at low concentrations of 25-50 mkM in and at low-temperature stress (5^0C) at fixed concentrations of 25-350 mkM; in the optimal mode of cultivation (25^0C) 2,6-*tert*-butyl phenol at low concentrations of 25-50 mkM and at low-temperature stress (5^0C) at fixed concentrations of 25-150 mkM exhibit growth-stimulating activity.

In *Dunaliella salina IPPAS D-294* cells grown under optimal conditions in the presence of the mineral medium of ionol, the biosynthesis of the total amount of chlorophylls (chlorophyll *a* up to 69%; chlorophyll *b* by 63%) and the synthesis of the total carotenoids up to 44% are suppressed, the ratio of chlorophyll *a* / chlorophyll *b* increases

[13] Alizadeh, G.I. The antioxidative activity of *Dunaliella* cells under low temperature stress / G.I. Alizadeh, A.R. Jalilova, Kh.Kh. Maharramova1 [et al.] // International Journal of Biopharmaceutical and Nanomedical Sciences, IJBNS, - 2013. 2 (1), - p. 74-78

[14] Alizadeh, G.I. Carotenogenesis in *Dunaliella* cells under stressed conditions, / G.I. Alizadeh, A.R. Jalilova, Kh.Kh. Maharramova [et al.] // European Journal of Biotechnology and Bioscience, - September 2017. 5 (5), - p. 41-46

and chlorophylls/carotenoids ratio[15] (Figure 3.2.1.). Under conditions of low-temperature stress, the biosynthesis of the amount of chlorophyll *a* and chlorophyll *b* increases. The ratio of chlorophyll *a* / chlorophyll *b* at low concentrations of ionol is suppressed, and at high concentrations it increases[16] (Figure 3.2.2.).

Under optimal conditions, the presence of 2,6 di-*tert*-butyl phenol in the mineral environment inhibits the biosynthesis of the total amount of chlorophylls (chlorophyll *a* and chlorophyll *b* up to 88%) and the synthesis of the total carotenoids up to 70%, the ratio of chlorophyll *a* / chlorophyll *b* and the ratio of chlorophyll also increase. /carotenoids.

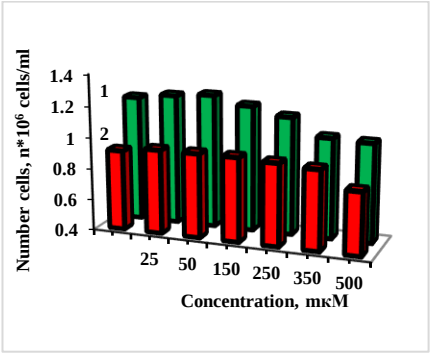

Figure 3.2.1. Growth dynamics of *Dunaliella salina IPPAS D-294* cells in the presence of various concentrations of ionol in a mineral medium under optimal (1) and low-temperature (2) cultivation modes. Temperature 27^0C, light intensity 16 W/m^2

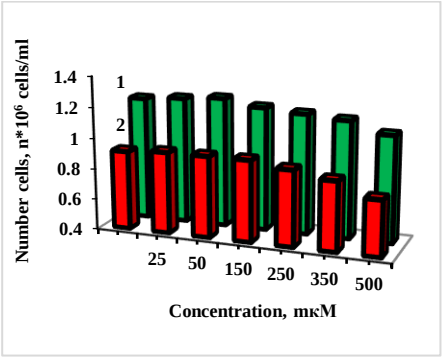

Figure 3.2.2. Growth dynamics of *Dunaliella salina IPPAS D-294* cells in the presence of various concentrations of 2,6 di-*tert*-butyl cresol in a mineral medium under optimal (1) and low-temperature (2) cultivation modes.Temperature 27^0C, light intensity 16 W/m^2

[15] Али-Заде, Г.И. Биосинтез пигментов в клетках *Dunaliella salina IPPAS D-294*, модифицированных ионолом при УФ-В облучении / Г.И. Али-Заде, А.Р. Джалилова, И.И. Алиев [и др.] // Научное обозрение. Биологические науки, - Москва: - 2020. №3, - с. 18-23.

[16] Али-заде, Г.И. Биосинтез пигментов в клетках *Dunaliella*, модифицированных ионолом, в условиях низкотемпературного стресса, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // Advances in Biology and Earth Sciences, - 2018. 3 (2), - p.152-159

Under conditions of low-temperature stress, the biosynthesis of the amount of chlorophyll *a* and chlorophyll *b* increases, the value of the chlorophyll *a* / chlorophyll *b* ratio increases, and the chlorophylls / carotenoids ratio is suppressed compared to control cells. Modification of cells with fixed concentrations of 25 - 500 mkM 2,6 di-*tert*-butyl cresol, photosynthetic release of oxygen is suppressed to 35-37%. Under conditions of low-temperature stress and under the influence of an antioxidant (25–500 mkM), functional activity is significantly reduced to 41–57%.

As a result of the presence of 2,6 di-*tert*-butyl phenol in the mineral medium at concentrations of 25–500 mkM, the functional activity is suppressed to 48%. Under conditions of low-temperature stress, the presence of 2,6 di-*tert*-butyl phenol in a mineral medium at concentrations of 25–500 mkM, compared with ionol, inhibits the functional activity of cells more pronouncedly - up to 64%[17].

3.3. Growth, biosynthesis of pigments and functional activity of cells of green microalgae *Dunaliella salina IPPAS D-294* at high salinity in optimal and low-temperature cultivation modes (3,0 M NaCl)

It is known that under the action of a high concentration of NaCl, the growth rate is reduced by 25%. This is due to the biosynthesis of glycerol and changes in the amount of carotenoids that protect the photosynthetic apparatus from photodamage under these conditions. In *Dunaliella salina IPPAS D-294* under optimal conditions (27^0C temperature, 16 W/m^2 light intensity, 1,5% CO_2 in the composition of the air mixture, 3,0 M NaCI in a mineral medium), the growth rate increases 3 times during the day. Under low-temperature stress, the bioproductivity of the cell population is suppressed by 2,5 times.

An increase in the salinity of the medium (3 M NaCl) leads to a decrease in the biosynthesis of chlorophylls *a* and *b*, and ultimately their total, while the amount of carotenoids remains at a fairly high

[17] Али-заде, Г.И. Биосинтез пигментов в клетках *Dunaliella*, модифицированных 2,6 ди-*трет*-бутил фенолом, в условиях низкотемпературного стресса, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова, И.И. Алиев // Экоэнергетика, - 2018. №3, - с. 20-26

level. The ratio of chlorophylls/carotenoids with increasing salinity from the optimal value (1,5 M NaCl) decreases and this affects the photosynthetic activity and the final bioproductivity.

Low-temperature stress somewhat increases the biosynthesis of chlorophylls and the amount of carotenoids. The ratios of the sum of chlorophylls and carotenoids in the optimal cultivation mode are 4,5 and 3,4, respectively, under conditions of low-temperature stress (Table 3.3.1.). Under low-temperature cultivation conditions, the functional activity is slightly lower (by 15%) than in cells grown under optimal conditions. The accumulation of total carotenoids under low-temperature stress leads to a decrease in the chlorophylls/carotenoids ratio and, thereby, to a decrease in the functional activity of *Dunaliella cells*.

Table 3.3.1

The content of pigments in *Dunaliella salina IPPAS D-294* cells grown at high salinity inoptimal and low-temperature conditions of cultivation

Mode cultivation	Chlorophyll *a* mg/l	Chlorophyll *b* mg/l	Sum Carotenoids mg/l	The ratio of chlorophylls/ carotenoids
K	3,13 ± 0,05	1,67 ± 0,05	1,06 ± 0,01	4,5
O	3,47 ± 0,05	1,99 ± 0,05	1,6 ± 0,01	3,4

Note: *optical density OD=0.8:* Temperature 27^0C, light intensity 16 W/m^2; K - blowing an air mixture into the photoreactors with a temperature of 25^0C; O-blowing into the photoreactors of an air mixture with a temperature of 5^0C (low-temperature stress)

3.4. Growth, pigment composition and functional activity of *Dunaliella salina IPPAS D-294* green microalgae cells modified with exogenous antioxidants under conditions of high salinity (3,0 M NaCl)

At high salinity of the mineral environment, ionol in the optimal cultivation mode (25^0C) - 25-350 mkM concentrations and at low-temperature stress (5^0C) - 25-500 mkM concentrations; 2,6 di-*tert*-butyl phenol in the optimal cultivation mode (25^0C) - 25-150 mkM concentrations and at low-temperature stress (5^0C) - 25-250 mkM concentrations exhibit growth-stimulating activity. In cells grown under optimal temperature conditions and high salinity of the mineral medium in the

presence of ionol, the biosynthesis of the total amount of chlorophylls is suppressed (chlorophyll *a* up to 82%; chlorophyll *b* by 85%) and the synthesis of the total carotenoids up to 6%, the ratio of chlorophyll *a* / chlorophyll *b* decreases as well as the ratio of chlorophylls / carotenoids[18] (figure 3.4.1). Under conditions of low-temperature stress, the biosynthesis of the amount of chlorophyll *a* is suppressed to 28%, and at concentrations of 25-350 mkM, the biosynthesis of chlorophyll *b* increases to 15%, and at a concentration of 500 mkM, it sharply drops by 65%. The amount of carotenoids increases by 46% and 39%, respectively, then is suppressed and remains at the control level[19] (figure 3.4.2). Under optimal conditions and high salinity of the mineral environment, the presence of 2,6 di-*tert*-butyl phenol inhibits the biosynthesis of the total amount of chlorophylls (chlorophyll *a* 70% and chlorophyll *b* up to 73%) and the synthesis of the total carotenoids up to 44%, the ratio of chlorophyll *a* / chlorophyll *b* increases and the chlorophylls/carotenoids ratio is suppressed.

Under conditions of low temperature stress and high salinity, the biosynthesis of chlorophyll *a* increases, and chlorophyll *b* is suppressed to 50%, and the value of the chlorophyll *a* / chlorophyll *b* ratio and the total carotenoids increase to 44%, the chlorophylls / carotenoids ratio is[20].

In the optimal mode (3,0M NaCl) in cells modified with various

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[18] Али-заде, Г.И. Устойчивости функциональной активности и антиоксидантной системы клеток *Dunaliella salina IPPAS D-294* модифицированных ионолом в оптимальных и условиях высокой солености / Г.И. Али-заде, А.Р. Джалилова, И.И. Алиев [и др.] // Веснік Гродзенскага дзяржаўнага ўніверсітэта імя Янкі Купалы. Серыя 5. Эканоміка. Сацыялогія. Біялогія, - Беларусь: - 2021. 11 (2), - с. 101-114

[19] Али-заде, Г.И. Биосинтез пигментов в клетках *Dunaliella salina IPPAS D-294,* модифицированных 2,6 ди-*трет*-бутил фенолом, в условиях высокой солености при оптимальном и низкотемпературном режимах культивирования / Г.И. Али-заде, А.Р. Джалилова, А.Э. Аббасова [и др.] // Экоэнергетика, - Баку: - апрель 2020. №2, - с. 129-134

[20] Али-заде, Г.И. Биосинтез пигментов в клетках *Dunaliella salina IPPAS D-294,* модифицированных 2,6 ди-*трет*-бутил фенолом, в условиях высокой солености при оптимальном и низкотемпературном режимах культивирования / Г.И. Али-заде, А.Р. Джалилова, А.Э. Аббасова [и др.] // Экоэнергетика, - Баку: - апрель 2020. №2, - с. 129-134

concentrations of 2,6 di-*tert*-butyl phenol (25-500 mkM), the functional activity of cells is suppressed to 20-28%, and in the low-temperature mode (3,0M NaCl) up to 71%. In the optimal cultivation mode (3,0 M NaCl), the photosynthetic activity of *Dunaliella salina IPPAS D-294* cells is suppressed when the cells are modified with 2,6 di-*tert*-butyl phenol of various concentrations.

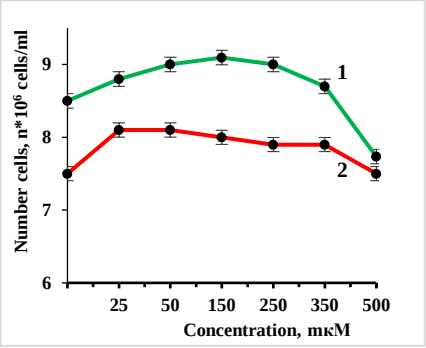

Figure 3.4.1. Dependence of the growth of *Dunaliella salina IPPAS D-294* cells on various concentrations of ionol in a mineral medium in the optimal (**1**) and low temperature (**2**) cultivation modes (3.0 M NaCl). Temperature 27^0C, light intensity 16 W/m^2

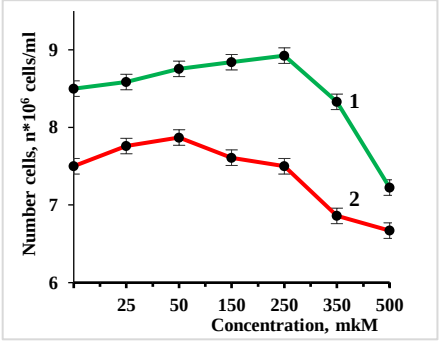

Figure 3.4.2. Dependence of the growth of *Dunaliella salina IPPAS D-294* cells on various concentrations of 2,6 di-*tert*-butyl cresol in a mineral medium in the optimal (**1**) and low temperature (**2**) cultivation modes (3,0 M NaCl). Temperature 27^0C, light intensity 16 W/m^2

IV CHAPTER. ENDOGENOUS ANTIOXIDANT ACTIVITY OF GREEN MICROALGAE DUNALIELLA SALINA IPPAS D-294 CELLS

4.1. Endogenous antioxidant activity of *Dunaliella salina IPPAS D-294* green microalgae cells modified with exogenous antioxidants (1,5 M NaCl)

In the optimal cultivation mode, in cells modified with 25-500 mkM concentrations of ionol, catalase activity increases to 55–65%; at 25–500 mkM concentrations of ionol, catalase activity increases to

a level of 40–42% higher than that of the control[21]. When cells are modified with 2,6-*tert*-butyl phenol (25–500 mkM), catalase activity in small doses of the antioxidant increases to 25–50%, and is suppressed with further increases in concentration. Under conditions of low-temperature stress, catalase activity in cells increases by 30-45%, at 350 and 500 mkM concentrations it remains at the level of 30-42% compared with the control suspension[22].

Nonspecific cell tolerance to the presence of synthetic antioxidants ionol and 2,6 di-*tert*-butyl phenol in the mineral growing medium and to low-temperature stress with the generation of reactive oxygen species contributes to an increase in catalase activity.

In *Dunaliella salina IPPAS D-294* cells in the optimal and low-temperature cultivation mode in the presence of 25-500 mkM concentrations of 2,6-*tert*-butyl cresol), the amount of malonic dialdehyde content is suppressed by 2 times[23]. *Dunaliella salina IPPAS D-294* cells in optimal and low-temperature cultivation modes in the presence of 25-500 mkM concentrations of 2,6-*tert*-butyl phenol, the amount of MDA content is suppressed up to 1,5 times[24]. Thus, the presence of 2,6 di-*tert*-butyl cresol and 2,6 di-*tert*-butyl phenol in the mineral medium is 24 hours of cultivation of cells of green microalgae *Dunaliella salina IPPAS D-294* significantly inhibits the generation of reactive oxygen species, thereby contributing to the suppression of the process

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[21] Джалилова, А.Р. Каталазная активность в клетках *Dunaliella salina İPPAS D-294*, модифицированных ионолом при оптимальном и низкотемпературном режимах культивирования / А.Р. Джалилова, Х.Х. Магеррамова, И.И. Алиев [и др.] // Advances in Biology & Earth Sciences, - 2019. 4 (2), - p.128-134

[22] Али-заде Г.И. Каталазная активность в клетках *Dunaliella salina IPPAS D-294,* модифицированных 2,6 ди-*трет*-бутил фенолом при оптимальном и низкотемпературном режимах культивирования, / Г.И. Али-заде, А.Р. Джалилова, Х.Х .Магеррамова [и др.] // - Баку: Ekologiya və su təsərrüfatı, - 2019. №1, - с. 6-12

[23] Али-заде, Г.И. Перекисное окисление липидов в клетках *Dunaliella* при низкотемпературном стрессе, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // Ekologiya və su təsərrüfatı, - Баку: - may 2014. №2, - s. 18-22

[24] Али-заде, Г.И. Перекисное окисление липидов в клетках *Dunaliella salina IPPAS D-294,* модифицированных 2,6 ди-*трет*-бутил фенолом при оптимальном и низкотемпературном режимах культивирования, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // - Баку: Экоэнергетика, - 2019. №1, - с. 19-24

of lipid peroxidation and ultimately reflected in the bioproductivity of microalgae.

4.2. Endogenous antioxidant activity of *Dunaliella salina IPPAS D-294* green microalgae cells modified with exogenous antioxidants (3,0 M NaCl)

Under conditions of high salinity (3,0 M NaCl) in the optimal cultivation mode in *Dunaliella salina IPPAS D-294* cells at 25–350 mkM concentrations of ionol, catalase activity increases to 30–35%, and is suppressed at 500 mkM concentrations (86%).

Under conditions of low-temperature stress, ionol concentration of 25 mkM promotes an increase in the activity of enzymatic catalase in cells by 65%, and at 50–500 mkM concentrations, it remains at a twofold value.

Under conditions of high salinity (3,0 M NaCl) and in the mode of optimal cultivation in cells at 25-50 mkM concentrations of 2,6-tert-butyl phenol, catalase activity increases to 55-65%, and at 150-500 mkM concentrations it is suppressed (86 %). Under conditions of low temperature stress and high salinity (3,0 M NaCl) in the presence of 25-50 mkM concentrations 2,6-*tert*-butyl phenol catalase activity increases by 50-80%, at 250:350 and 500 mkM concentrations, remains at the level of 48-50%[25]. 500 mkM concentration of ionol, the amount of malon dialdehyde content is suppressed by 35-60%. Under low-temperature stress at concentrations of 150–500 mkM ionol, the amount of malon dialdehyde in cells decreases by 4–20%, and 25 mkM and 50 mkM antioxidant concentrations do not affect the LPO process in cells.

At high salinity of the mineral medium and under conditions of optimal cultivation in the presence of 25-500 mkM concentrations of 2,6-*tert*-butyl phenol, the amount of MDA content increases by 35-60%. Under low-temperature stress, 2,6 di-*tert*-butyl phenol increases the amount of malon dialdehyde in cells by 32%. In the presence of

[25] Али-заде, Г.И. Каталазная активность в клетках *Dunaliella salina IPPAS D-294*, модифицированных 2,6 ди-*трет*-бутил фенолом в условиях высокой солености при оптимальном и низкотемпературном режимах культивирования / Али-заде Г.И., Джалилова А.Р., Халилов Р.И., Сулейманова Л.М. // Академия Наук Республики Узбекистан. Узбекский Биологический Журнал. – Ташкент: - 2021. №4, - с. 30-35

150 and 500 mkM concentrations of 2,6 di-*tert*-butyl phenol in a mineral growing medium and under low-temperature stress conditions, there was no effect on the process of LPO[26].

V CHAPTER. FUNCTIONAL STABILITY OF GREEN MICROALGAE *DUNALIELLA SALINA IPPAS D-294* CELLS

5.1. Functional resistance of *Dunaliella salina IPPAS D-294* green microalgae cells to acute doses of UV-B radiation modified with exogenous antioxidants (1,5 M NaCl)

The results of photosynthetic oxygen evolution, irradiated with various acute doses of UV-B radiation by *Dunaliella salina IPPAS D-294* control cells, and cells modified with 25 mkM and 50 mkM concentrations of ionol showed that, in pre-irradiated $2{,}2 \cdot 10^2$ J/m^2, $3{,}75 \cdot 10^2$ J/m^2 and $6 \cdot 10^2$ J/m^2 with acute doses of control cells, the relative amount of released photosynthetic oxygen, respectively, sharply decreases by 30-32%, 40% and 38%. Cells grown in the presence of 25 mkM concentration of 2,6 di-*tert*-butyl cresol and irradiated with $2{,}2 \cdot 10^2$ J/m^2, 3,75 10^2 J/m^2 and $6{,}0 \cdot 10^2$ J/m^2 with acute doses of UV-B radiation showed high stability of the photosynthetic apparatus 95%, 96% and 77% respectively; in the presence of 50 mkM concentration, the tolerance of the photosynthetic apparatus remains at a high level[27]. Under conditions of low-temperature stress in pre-irradiated $6{,}0 \cdot 10^2$ J/m^2, $10 \cdot 10^2$ J/m^2 and $12 \cdot 10^2$ J/m^2 with acute doses of control cells, the relative amount of released photosynthetic oxygen sharply decreases by 10%, 29% and 68% respectively. Cells grown in the presence of 25 mkM concentration of 2,6 di-*tert*-butyl cresol and irradiated with

[26] Али-заде, Г.И. Перекисное окисление липидов в клетках *Dunaliella salina IPPAS D-294* модифицированных 2,6 ди-*трет*-бутил фенолом в условиях высокой солености при оптимальном и низкотемпературном режимах культивирования / Г.И. Али-заде, А.Р. Дажалилова, И.И. Алиев [и др.] // Академия Наук Республики Узбекистан. Узбекский Биологический Журнал, - Ташкент: - 2021. №2, с. 7-11

[27] Али-заде, Г.И. Функциональная устойчивость клеток *Dunaliella* к острым дозам УФ-В излучения, модифицированных ионолом, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // Ekologiya və su təsərrüfatı, - Баку: - 2017. №5, - с. 7-11

$6{,}0 \cdot 10^2$ J/m^2, $10 \cdot 10^3$ Erg/mm^2 and $12 \cdot 10^2$ J/m^2 with acute doses of UV-B radiation showed high the stability of the photosynthetic apparatus is 100%, 71% and 68%; in the presence of 50 mkM concentration of 100%, 95% and 82% respectively. As a result of studies, the protective role of the synthetic antioxidant 2,6 di-*tert*-butyl cresol under the action of UV-B light was revealed[28].

In control cells pre-irradiated in the range of $2{,}2 \cdot 10^2$ J/m^2 – $6{,}0 \cdot 10^2$ J/m^2 with acute doses, photosynthetic activity is sharply suppressed.

The antioxidant 2,6 di-*tert*-butyl phenol at a concentration of 25 mkM exhibits a weak protective function. Cells grown in the presence of 50 mkM concentration of 2,6 di-*tert*-butyl phenol show a gradual decline in functional activity with increasing acute dose of UV-B radiation.

Under conditions of low-temperature stress, when irradiated with various acute doses of UV-B light in pre-irradiated people in the range of $6{,}0 \cdot 10^2$ J/m^2, $10 \cdot 10^2$ J/m^2 - $12 \cdot 10^2$ J/m^2 with acute doses of control cells, the relative amount of released photosynthetic oxygen is sharply suppressed. Cells grown in the presence of 25 mkM concentration, irradiated with $6{,}0 \cdot 10^2$ J/m^2, $10 \cdot 10^2$ J/m^2 and $12 \cdot 10^2$ J/m^2 with acute doses of 2,6 di-*tert*-butyl phenol demonstrates a smooth decline in functional activity with an increase in the acute dose of UV-B radiation by 98%, 98% and 58% in presence 50 mkM concentrations of 96%, 71% and 30%, respectively[29].

5.2. Functional stability of green cells microalgae *Dunaliella salina IPPAS D-294*to acute doses of UV-B radiation modified with exogenous antioxidants (3,0 M NaCl)

In the optimal mode and under conditions of high salinity of the mineral environment with preliminary irradiation of $15 \cdot 10^2$ J/m^2,

[28] Али-заде, Г.И. Функциональная активность и УФ-В толерантность клеток *Dunaliella*, модифицированные синтетическими антиоксидантами в условиях низкотемпературного стресса, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // AMEA-nın xəbərləri, Biologiya elmləri seriyası, - Bakı: - 2017, 72 (2), - s. 106-113

[29] Али-заде, Г.И. Функциональная устойчивость клеток *Dunaliella* к острым дозам УФ-В излучения, модифицированные синтетическими антиоксидантом 2,6 –ди-*трет*-бутил фенолом, / Г.И. Али-заде, А.Р. Джалилова, И.И. Алиев [и др.] // Экоэнергетика, - Баку: - 2018. №1, - с. 26-34

$18•10^2$ J/m^2 and $21•10^2$ J/m^2 with acute doses of control cells, the relative amount of released photosynthetic oxygen is reduced by only 94%, 74% and 35%, respectively. Cells grown in the presence of 25 mkM concentration of 2,6 di-*tert*-butyl cresol and irradiated with $15•10^2$ J/m^2, $18•10^2$ J/m^2 and $21•10^2$ J/m^2 with acute doses of UV-B radiation did not show the stability of the photosynthetic apparatus 86%, 74% and 37%; in the presence of 50 mkM concentrations of 99%, 80% and 56%, respectively (Figure 5.2.1).

Under conditions of low temperature stress and high salinity in pre-irradiated $15•10^2$ J/m^2, $18•10^2$ J/m^2 and $21•10^2$ J/m^2 with acute doses of control cells, the relative amount of released photosynthetic oxygen sharply decreases by 68%, 60% and 55%, respectively.

Cells grown in the presence of 25 mkM concentration of 2,6 di-*tert*-butyl cresol and irradiated with $15•10^2$ J/m^2, $18•10^2$ J/m^2 and $21•10^2$ J/m^2 with acute doses of UV-B radiation showed high stability of the photosynthetic apparatus by 90%, 86% and 46%; in the presence of 50 mkM concentrations of 95%, 90% and 62%, respectively (Figure 5.2.2). In the optimal cultivation mode and under conditions of high salinity, in pre-irradiated in the range of $15•10^2$ J/m^2 - $21•10^2$ J/m^2 with acute doses of control cells, the relative amount of released photosynthetic oxygen is sharply suppressed by 59%, 49% and 47%, respectively[30,31].

In the presence of 25 mkM concentration of 2,6 di-*tert*-butyl phenol and irradiated $15•10^2$ J/m^2, $18•10^2$ J/m^2 and $21•10^2$ J/m^2 with acute doses of UV-B radiation showed high stability of the photosynthetic apparatus 87%, 76% and 59%; in the presence of 50 mkM concentration of 2,6 di-*tert*-butyl phenol, a gradual decrease in functional activity is observed with an increase in the acute dose of UV-B radiation.

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[30] Али-заде, Г.И. Устойчивость функциональной активности клеток *Dunaliella* к УФ-В излучению, модифицированные синтетическим антиоксидантом ионолом в условиях низкотемпературного стресса и высокой солености, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // Ekologiya və su təsərrüfatı, - Баку: - 2018. №5, - с. 3-9

[31] Джалилова, А.Р. Резистентность функциональной активности модифицированных ионолом клеток *Dunaliella salina IPPAS D-294*, к действию острых доз УФ-В радиации в оптимальных и в условиях высокой солености // - Ташкент: Узбекский Биологический Журнал, – 2022. №1. - с. 9-14

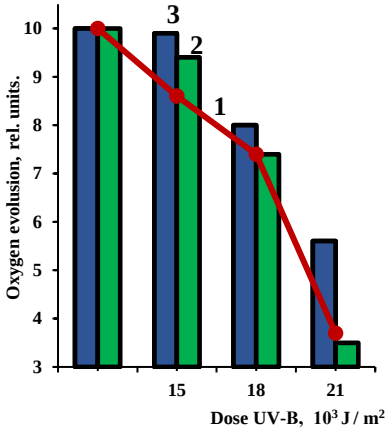

Figure 5.2.1. Photosynthetic release of oxygen by control cells and cells grown in a medium with high salinity and various concentrations of 2,6 di-*tert*-butyl cresol upon irradiation with acute doses of UV-B light: **1**- control; **2**- 25 mkM 2,6 di-*tert*-butyl cresol; **3**- 50 mkM 2,6 di-*tert*-butyl cresol. Temperature 40^0C, light intensity 100 W/m^2

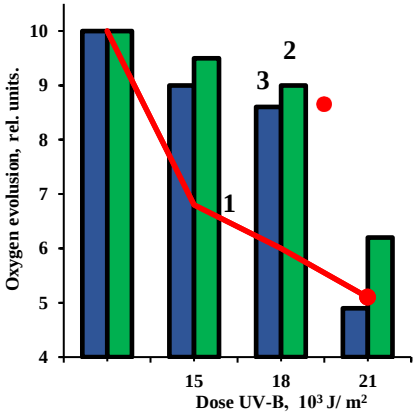

Figure 5.2.2. Photosynthetic release of oxygen by control cells and cells grown under conditions of low temperature stress and high salinity in a medium with various concentrations of 2,6 di-*tert*-butyl cresol upon irradiation with acute doses of UV-B light: **1**- control; **2**- 25 mkM 2,6 di-*tert*-butyl cresol; **3**- 50 mkM 2,6 di-*tert*-butyl cresol. Temperature 40^0C, light intensity 100 W/m^2

Under conditions of low temperature stress and high salinity in pre-irradiated $15 \cdot 10^2$ J/m^2, $18 \cdot 10^2$ J/m^2 and $21 \cdot 10^2$ J/m^2 with acute doses of control cells, the relative amount of photosynthetic oxygen release is suppressed by 74%, 67% and 60%, respectively.

In the presence of 25 mkM concentration of 2,6 di-*tert*-butyl phenol and irradiated $15 \cdot 10^2$ J/m^2, $18 \cdot 10^2$ J/m^2 and $21 \cdot 10^2$ J/m^2 with acute doses of UV-B radiation showed high stability of the photosynthetic apparatus 87%, 76% and 59%; in the presence of 50 mkM concentrations of 90%, 80% and 68%, respectively.

Thus, the synthetic antioxidants ionol and 2,6 di-*tert*-butyl phenol protect the functional activity of cells from acute doses of UV-B radiation differently. The protective function of 2,6 di-*tert*-butyl cresol

exceeds that of 2,6 di-*tert*-butyl phenol[32,33].

Thus, cells grown under the low-temperature cultivation mode exhibit higher levels of stability of functional activity under the action of various acute doses of UV-B radiation.

Indicators of the functional stability of cells under the action of high salinity, low temperature stress can be interpreted by two mechanisms: the first increase in endogenous antioxidant systems under the action of low temperature stress due to an increase in the amount of reactive oxygen species; the second is known as cross-adaptation of cells under successive actions of various stressors.

CONCLUSIONS

1. It has been established that bioproductivity of *Dunaliella salina IPPAS D-294* suspensions grown under optimal conditions (1,5 M NaCl) and high salinity (3,0 M NaCl) of the mineral medium is suppressed under low-temperature stress conditions.
2. A high plateau of growth in the biosynthesis of total carotenoids by *Dunaliella salina IPPAS D-294* cells was revealed in the low-temperature stress mode, under conditions of optimal (1,5 M NaCl) and high salinity (3,0 M NaCl) of the mineral environment, which leads to the suppression of the functional activity of algae.
3. It has been established that the presence of synthetic antioxidants (25-500 mkM) in a mineral medium, at optimal (1,5 M NaCl) and high salinity (3,0 M NaCl), leads to stimulation of the growth of the culture of control cells (25-350 mkM), and in under low-temperature stress, the range of concentrations of synthetic antioxidants that

[32] Джалилова, А.Р. Ответные реакции антиоксидантной системы и функциональная устойчивость к УФ-В излучению клеток *Dunaliella salina IPPAS D-294* модифицированных 2,6 ди-*трет*-бутил фенолом в оптимальных условиях и при высокой солености // - Баку: Advances in Biology & Earth Sciences, - 2022. 7 (1). - p. 59-71

[33] Али-заде, Г.И. Устойчивость функциональной активности клеток *Dunaliella* к УФ-В излучению, модифицированные синтетическим антиоксидантом 2,6 ди-*трет*-бутил фенолом в условиях низкотемпературного стресса и высокой солености, / Г.И. Али-заде, А.Р. Джалилова, Х.Х. Магеррамова [и др.] // Advances in Biology and Earth Sciences, - 2018. 3 (3), - p. 257-263

stimulate population growth expands (25-500 mkM). It is possible that the synthetic antioxidant 2,6 di-*tert*-butyl cresol (ionol) and the new 2,6 di-*tert*-butyl phenol imitate and even act as a growth-regulating agent.

4. In a comparative study of the endogenous antioxidant system of *Dunaliella salina IPPAS D-294* cells modified with synthetic antioxidants (25-500 mkM) in the optimal salinity (1,5 M NaCl) of the mineral medium, a twofold increase in catalase activity and a suppression of the content of malon dialdehyde up to 50% were revealed under the action of low positive temperatures on the cell population.
5. It was found that modification of cells with different concentrations (25-500 mkM) of synthetic antioxidants in the regime of optimal and low-temperature stress, at high salinity (3,0 M NaCl) of the mineral environment leads to changes in the activity of endogenous antioxidants: an increase in catalase activity and a decrease in the content of malon dialdehyde.
6. A comparative study of the functional activity of *Dunaliella salina IPPAS D-294* cells modified with synthetic antioxidants (25-50 mkM) in the optimal cultivation mode and low-temperature stress showed that the resistance of the population to acute doses of UV-B radiation doubles when exposed to low positive temperatures on the population cells.
7. It was found that the increase in salinity (3,0 M NaCl) in the mineral medium and the modification of cells with synthetic antioxidants shifts the functional resistance to higher values of UV-B radiation compared to (1,5 M NaCl).

PRACTICAL RECOMMENDATIONS

1. To activate the bioproductivity of the *Dunaliella salina IPPAS D-294* cell culture, the exogenous antioxidant 2,6-di-*tert*-butyl cresol and 2,6-di-*tert*-butyl phenol are recommended as promising and effective agents, reliable and affordable growth activators (regulators);
2. To increase the functional resistance of microalgae to subsequent exposure to various acute doses of UV-B radiation, it is recommended to increase the intracellular biosynthesis of the total carotenoids associated with low-temperature stress (5^0C) and an increase in the salinity of the mineral environment (3,0M NaCl);
3. For genetic monitoring, when assessing the biological consequences of the action of abiotic environmental factors, it is recommended to use cells of green microalgae *Dunaliella salina İPPAS D-294* as promising test objects;
4. The results of the studies carried out are recommended to be used in the development of the basic principles for assessing the functional resistance of unicellular algae to abiotic factors.

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