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The stability of functional activity in Dunaliella Salina IPPAS-294 cells modified by synthetic antioxidants in conditions of low temperature stress and high salinity

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Abstract
On the basis of obtained results, has been shown that, the modification of cells by synthetic antioxidants within 24 hours in conditions of low temperature stress and high salinity increases amount of synthesized carotenoids, decreases POL process, and also increases catalase activity. It became clear that, Dunaliella cells modified by synthetic antioxidants in conditions of low temperature stress and high salinity, shows higher functional stability against the influence of further various acute doses of UV-B irradiation.

Keywords: dunaliella cells, salinity, low temperature stress, synthetic antioxidants, UV-B irradiation

1. Introduction
There are large number of synthetic compounds, which regulate plant growth and development in exogenous use. Compounds, which slow down plant growth by inhibition of cell stretching and their deletion, are called growth retardants, are those, which execute growth-stimulating activities [1, 7]. Significant interest presents investigations of features of antioxidant activities such as ionol and its analogue 2, 6 di-tret-butyl, which belong to the class of spatially hindered phenols [3]. For more efficient use of antioxidants, it is necessary to link chemistry and biology of antioxidants, also the dependence of biological activity of antioxidants, their features as inhibitors of radical reactions, and also their efficient concentrations [1, 6]. The possibility of using antioxidant in planting as growth stimulation was actively investigated [4]. It is also known that, synthetic antioxidants in high concentrations begin to act in opposite direction and do not brake, on the contrary, accelerate free radical reactions [8].
There is very little information on the synthetic antioxidant influence and their antiradical features in green algae. Due to that, the purpose of our work was the investigation influence of various concentrations of synthetic antioxidants 2,6 di-tret-butyl cresol (BHT- classic synthetic antioxidant) and 2,6 di-tret-butyl phenol on the growth, activity of endogen antioxidant systems of Dunaliella algae and their UV protection activity in cells in conditions of low temperature stress and high salinity.

2. Materials and Methods
As investigation object was used halophylic green algae Dunaliella Salina IPPAS-294, taken from the saline lake Masazir located on the north eastern part of Baku in Azerbaijan.
The algae were grown at 27^0C temperature3v in glass photo reactors (250ml), in the installation for growing unicellular algae. Mineral medium contained (g\l): NaCl-175, 5 (3,0M), KNO_3 - 5,0; KH_2PO_4 -1,25; $MgSO_4$-50; $FeSO_4$ - 0,009 microelement solutions(mg\l) – $Ca(NO_3)_2$ • H_2O – 735; $ZnSO_4$ • $7H_2O$ – 615; $(NH_4)MoO_4$ - 100; $MnCl_2$• $4H_2O$ – 180. The cell suspension in photo reactors was irradiated by white light (16Wt\m^2) within 24 hours and permanently purged with mixture (air+1,5% CO_2) at 25^0 C temperature. The cells were cultivated within 24 hours in intensive-accumulated cultivation regime and irradiated by counting cell number in Qoryayev chamber under the microscope or by nephelometric measurement of optic suspension density in photoelectrocolorimeter.
The content of pigments in cellular extracts (100% acetone) was measured in spectrophotometer and counted on base of Wettshtain coefficient [2].
To measure the photosynthetic cell activity, of grown algae were precipitated by centrifugation (30000 rev\min.) within 10 min. at room temperature and transferred into newly-made mineral medium. The suspension density in cells was led to 10^6 cells\ml (optic density OD=0,8). The speed of oxygen evolution in cells was measured in polarographic installation, using platinum electrode Klark, lightening the suspension in thermo stated volume, saturating the intensity by white light (100Wt\m^2).
In order to measure the catalase activity in cells, the suspension precipitated by centrifugation (30000 rev\min.). the sediment was transferred into a mortar with 0,5g $CaCO_3$ was added 5 ml distilled water and triturated into homogenous mass. Then gained mass quantitatively transferred into a glass with 50 ml capacity till the mark and infused with periodic shaking 3-4 hours. Within that time, enzyme extraction happens in plant material. After infusion the suspension was filtered in dry glass. Catalase activity was measured by geometric method, which based on determination of volume after adding into the aqueous extract of plants, containing catalase, hydrogen peroxide [5].
The evaluation degree of lipid peroxidation (POL) was carried out by the method of determining MDA content in Dunaliella Salina cells – method based on the reactions with thibarbituric acids. The cell suspension (35ml) was centrifuged 30000 rev\min. within 10 minutes. The resulting sediment was homogenized in 20 ml 0, 1% TCA. Homogenate was centrifuged at 30000 rev\min. within 10 minutes. To the 1 ml supernatant was added4 ml 20% TCA,

containing 0, 5% TBA. The mixture was heated in water bath at 95^0Cwithin 30 minutes and immediately cooled in running water. After centrifugation of mixture at 30000 rev\min. within 10 minutes was determined optic density of supernatant at 532 nm [9].

3. Results and Discussions

In figure 1 has been presented the growth dependence of Dunaliella Salina IPPAS-294 cells in various concentrations of 2,6 di-tret-butyl cresol(BHT) [1] and 2,6 di-tret-butyl phenol [2] in mineral medium, in conditions of low temperature stress and high salinity. As seen in the figure, the presence of BHT and 2, 6 di-tret-butyl phenol in the medium visibly influence on the culture growth. Thus, BHT and 2,6 di-tret-butyl phenol at concentrations 25mkM and 350mkM in mineral medium of BHT cell growth increases up to 8-5%, but in the presence of 2,6 di-tret-butyl phenol at concentrations 25-150 mkM bioproductivity increases up to 3,5-1,5% compared to control suspensions. At concentrations 500 mkM in mineral medium in conditions of low temperature stress and high salinity (3,0 MNaCl) BHT influence on growth remains on control level, but in the presence of 2,6 di-tret-butyl phenol at concentrations 250 mkM control level remains. Further increase in the content of 2, 6 di-tret-butyl phenol in mineral medium at concentrations 350-500 mkM was observed the suppression 8,5% and 11% relatively of culture growth during 24 hourly cultivation in intensive-accumulative regime, conditions of low temperature stress and high salinity (3,0 MNaCl). Expressed growth stimulating activity at concentrations 25-500mkM of BHT and 2, 6 di-tret-butyl phenol in conditions of low temperature stress and high salinity makes those antioxidants perspective and effective resources being available and reliable regulation (activation) of culture growth in Dunaliella Salina IPPAS-294 cells.

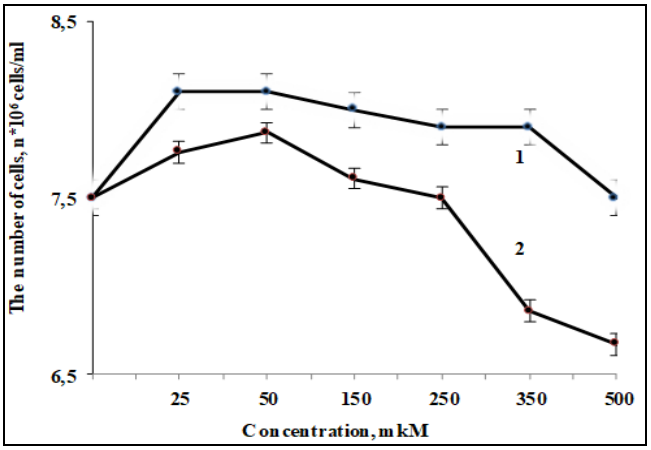


Fig 1: The dependence of population growth Dunaliella Salina IPPAS-294 cells at various concentrations of 2,6 di-tret-butyl cresol (BHT) (1) and 2,6 di-tret-butyl phenol (2) in mineral medium in conditions of low temperature stress and high salinity (3,0 MNaCl)

The number of cells n* 10^6 cells\ml

Revealed by us growth and development stimulation of the algae by ionol activity in comparison with the activity of 2,6 di-tret-butyl phenol, which is similar to ionol in structure, bur does not have methyl groups, also occurred to be an effective antioxidant and physically active. This joint has similar activity with BHT, grown in presence of 25-250mkM of 2,6 di-tret-butyl phenol in conditions of low temperature stress and high salinity, was observed growth stimulation of the algae in intensive cultivation (fig1. curve.1.). Concentrations of 350mkM and 500mkM within 24 hours cultivation and in conditions of low temperature stress and high salinity, suppress bioproductivity of cells. So, the presence of 2,6 di-tret-butyl phenol in mineral medium with various concentrations due to growth indicators acts like an antioxidant similar to ionol. It is not excluded that, the synthetic antioxidant 2,6 di-tret-butyl cresol(ionol) and its analogue 2,6 di-tret-butyl phenol imitate and also play the role of growth stimulating agents.

In order to identify functional activity of Dunaliella Salina IPPAS-294 in cell modification of algae within 24 hours at various concentrations of synthetic antioxidants ionol and 2,6 di-tret-butyl phenol in mineral medium in conditions of low temperature stress and high salinity, have showed that, ionol and 2,6 di-tret-butyl phenol in investigated range reduces the photosynthetic oxygen evolution of cell suspension.

In figure 2 have been presented the results of photosynthetic oxygen evolution in Dunaliella Salina IPPAS-294 cells grown at various concentrations of 2,6 di-tret-butyl cresol (BHT) (1) and 2,6 di-tret-butyl phenol (2) in mineral medium in conditions of low temperature stress and high salinity. As seen in the figure, the investigation of the photosynthetic oxygen evolution grown in regimes of low temperature stress and high salinity of cultivation. (fig.2.curve.1.) at concentrations 25; 50 and 150 mkM functional cell activity is slightly suppressed 98%; 95% and 91% accordingly. The increase of synthetic antioxidant concentrations up to 250 mkM; 350 mkM and 500 mkM in mineral medium suppresses functional activity 38%; 46% and 71% accordingly. The investigation of the photosynthetic oxygen evolution in Dunaliella Salina IPPAS-294 cells grown at various concentrations of 2,6 di-tret-butyl phenol in mineral medium in conditions of low temperature stress and high salinity, has showed that, the concentrations (25-500mkM) of 2,6 di-tret-butyl phenol lead to suppression of functional activity of Dunaliella cells. In this case, observed suppression of function by 2,6 di-tret-butyl phenol significantly differs from an antioxidant BHT, so at the concentrations 25 mkM and 50 mkM suppression of functional activity in cells is slightly 7% and 8% accordingly.

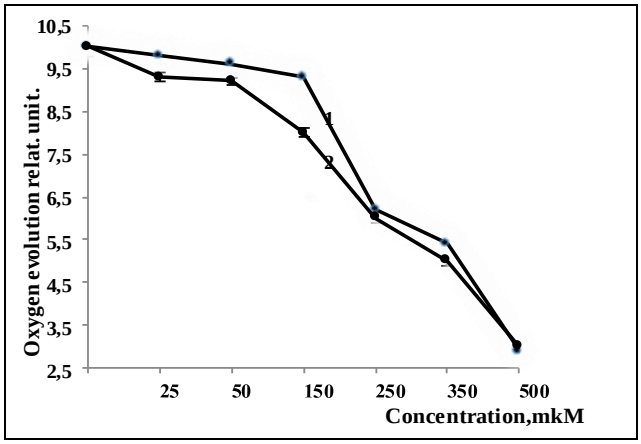


Fig 2: Photosynthetic oxygen evolution in Dunaliella Salina IPPAS-294 cells grown at various concentrations of 2,6 di-tret-butyl cresol (BHT) (1) and 2,6 di-tret-butyl phenol (2) in mineral medium in conditions of low temperature stress and high salinity (3,0 MNaCl)

Temperature 40%, light intensity 100 Wt\m^2

The increase of concentrations up to 150 mkM; 250 mkM leads to rapid 20% and 40% reduction of photosynthetic oxygen evolution in cells, but concentrations 350 mkM and 500 mkM to 20% and 40%, which is not observed in BHT investigations. Though, plant cells usually have high level of antioxidative activity. We would like to investigate on what level synthetic antioxidants used in growth culture within 24 hours cultivation in conditions of low temperature stress and high salinity can influence on endogen low molecular (caratenoids) and high molecular (catalase) antioxidants, also the process of lipid peroxidation.

In table 1 has been presented the growth indications, pigment formation, catalase activity and amount of MDA formation in Dunaliella cells in control and also during processing the cells by synthetic antioxidant 2,6 di-tret-butyl cresol (BHT) at various concentrations during 24 hourly cultivation in conditions of low temperature stress and high salinity. As seen in the schedule, the modification of cell suspension by BHT at concentrations 25 - 500 mkM leads to the increase of endogen catalase activity up to 50% compared to control cells.

Table 1: The growth indications, pigment formation, catalase activity and amount of MDA formation in Dunaliella cells in control and also during processing the cells by synthetic antioxidant 2,6 di-tret-butyl cresol (BHT) in conditions of low temperature stress and high salinity (3,0 MNaCl)

	Growth, OD		Catalase activity, mκM H_2O_2 ml^{-1} min^{-1}.				The amount of pigments, mg/l.			The content of MDA, mol/graw weight
			5	10	15	20	C_a	C_b	C_{car}	
K	0,3	0,75±0,03	0,4	0,9	1,2	1,9	3,47±0,05	1,99±0,05	1,6±0,1	0,98*10^{-3}±0,05
O_1	0.3	0,81±0,03	0,6	1,3	2, 0	2,15	3,68±0,05	2,11±0,05	2,34±0,1	0,85*10^{-3}±0,05
O_2	0.3	0,81±0,03	0,6	1,35	2,35	2,6	3,35±0,05	2,09±0,05	2,23±0,1	0,82*10^{-3}±0,05
O_3	0.3	0,8±0,03	0,7	1,1	2,45	2,85	3,18±0,05	2,17±0,05	1,57±0,0	0,85*10^{-3}±0,05
O_4	0.3	0,79±0,03	0,65	1,2	2,6	2,8	3,12±0,05	2,3±0,05	1,62±0,1	0,88*10^{-3}±0,05
O_5	0.3	0,79±0,03	0,75	1,4	2,5	2,7	3,12±0,05	2,3±0,05	1,62±0,1	0,75*10^{-3}±0,05
O_6	0.3	0,75±0,03	0,35	1,65	2,4	2,7	2,5±0,05	1,3±0,05	1,62±0,1	0,58*10^{-3}±0,05

Note: optic density OD=0,8; Temperature 27^{0}C, light intensity 16 Wt\m^2; C-control; O_1 - treatment 2,6 di-tret-butyl cresol (BHT) (25mkM); O_2 - treatment 2,6 di-tret-butyl cresol (50mkM); O_3 - treatment 2,6 di-tret-butyl cresol (150mkM); O_4 - treatment 2,6 di-tret-butyl cresol (250 mkM); O_5 - treatment 2,6 di-tret-butyl cresol (350 mkM); O_6 - treatment 2,6 di-tret-butyl cresol (500mkM)

The treatment of cell suspension with ionol at concentrations 25 mkM; 50mkM and 150mkM; synthesis of carotenoid amount increases up to 40% and 46%accordingly compared to control. Only at concentrations 250mkM 2% suppression of synthesis of carotenoid amount is observed, but at concentrations 350-500 mkM carotenoid synthesis reaches the control level. The treatment of cells with BHT increases chlorophyll biosynthesis «a» and «b». The evaluation intensity processes of lipid peroxidation (POL) were also conducted in conditions of cell modification by synthetic antioxidant BHT on the stage of algae development in intensive-accumulative regime of cultivation in conditions of low temperature stress and high salinity. The intensity indications of lipid peroxidation processes of cells defining in content MDA, at concentrations 25-500 mkM decrease compared to control.

So, the modification of Dunaliella cell suspension by various concentrations of BHT during 24 hours cultivation in conditions of low temperature stress and high salinity leads to the changes of endogen antioxidant system, which influence on functional activity and bioproductivity of algae.

The second synthetic antioxidant 2,6 di-tret-butyl phenol was also investigated on the range of concentration 25-500 mkM in medium during 24 hours cultivation of algae suspension in conditions of low temperature stress and high salinity.

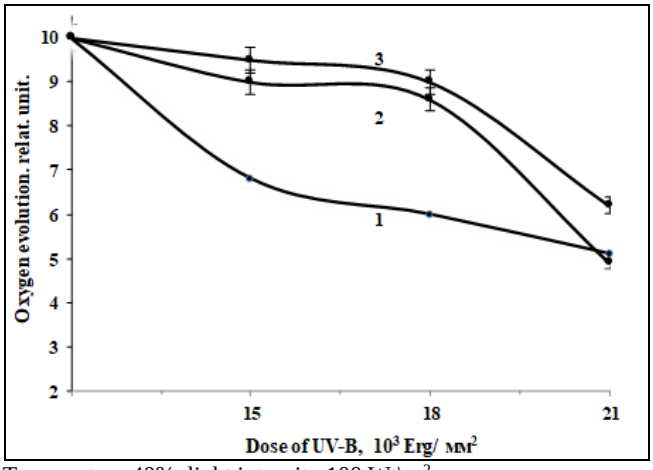
In table 2 have been presented the growth indications, pigment formation, catalase activity and amount of MDA formation in Dunaliella cells in control and by treating cells with antioxidant 2,6 di-tret-butyl phenol in conditions of low temperature stress and high salinity. As seen in the schedule, the modification of cells by 2,6 di-tret-butyl phenol leads to the increase of endogen catalase activity up to 44%. Also at concentrations 25-500 mkM to the increase of indications of carotenoid biosynthesis in regard to control cells. The increase of concentrations of synthetic antioxidant 2,6 di-tret-butyl phenol in mineral medium on range 250-500 mkM decreases chlorophyll biosynthesis «a» and «b».

Table 2: The growth indications, pigment formation, catalase activity and amount of MDA formation in Dunaliella cells in control and by treating cells with antioxidant 2,6 di-tret-butyl phenol in conditions of low temperature stress and high salinity (3,0 MNaCl)

	Growth, OD		Catalase activity, mκM H_2O_2 ml^{-1} min^{-1}.				The amount of pigments, mg/l.			The content of MDA, mol/graw weight
			5	10	15	20	Ca	Cb	C_{car}	
K	0,3	0,75±0,03	0,4	0,95	1,25	2,0	3,47±0,05	1,99±0,05	1,6±0,1	0,93*10^{-3}±0,05
O_1	0.3	0,78±0,03	0,6	1,35	1,9	2,6	4,58±0,05	1,81±0,05	1,89±0,1	0,8*10^{-3}±0,05
O_2	0.3	0,79±0,03	0,65	1,7	2,3	2,9	4,61±0,05	1,7±0,05	2,0±0,1	0,75*10^{-3}±0,05
O_3	0.3	0,76±0,03	0,55	1,55	2,4	2,8	4,1±0,05	1,6±0,05	2,14±0,0	0,68*10^{-3}±0,05
O_4	0.3	0,75±0,03	0,55	1,45	2,3	2,8	3,5±0,05	1,7±0,05	2,3±0,1	0,63*10^{-3}±0,05
O_5	0.3	0,69±0,03	0,58	1,6	2,4	2,8	2,95±0,05	1,14±0,05	2,13±0,1	0,65*10^{-3}±0,05
O_6	0.3	0,67±0,03	0,45	1,1	2,3	2,8	2,43±0,05	0,945±0,05	1,81±0,1	0,68*10^{-3}±0,05

Note: optic density OD=0,8; Temperature 27^{0}C, light intensity 16 Wt\m^2; C-control; O_1 - treatment 2,6 di-tret-butyl phenol (25mkM); O_2 - treatment 2,6 di-tret-butyl phenol (50mkM); O_3 - treatment 2,6 di-tret-butyl phenol (150mkM); O_4 - treatment 2,6 di-tret-butyl phenol (250 mkM); O_5 - treatment 2,6 di-tret-butyl phenol (350 mkM); O_6 - treatment 2,6 di-tret-butyl phenol (500mkM).

The (POL) indications, defining by content MDA, at concentrations 25-500 mkM remains below the control level. The main purpose in investigations was the explanation stability limits of Dunaliella cells and also in cells modified by synthetic antioxidants 2,6 di-tret-butyl cresol and 2,6 di-tret-butyl phenol with concentrations 25 and 50 mkM in conditions of low temperature stress and high salinity under the influence of various acute doses of UV-B irradiation.

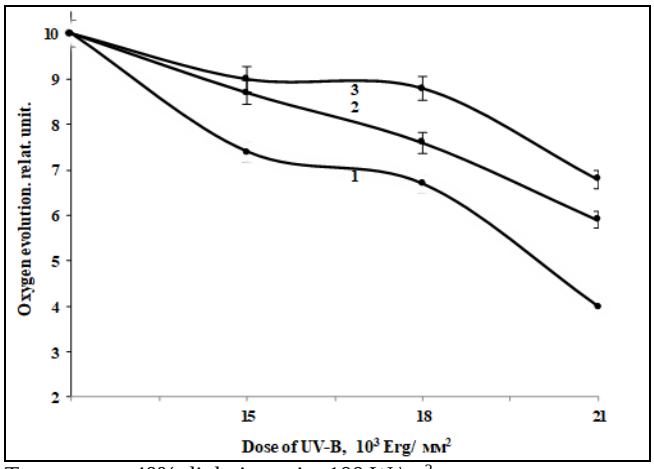


Temperature 40%, light intensity 100 Wt\m^2

Fig 3: Photosynthetic oxygen evolution of control Dunaliella cells and the cells grown in medium with various concentrations of 2, 6 di-tret-butyl cresol (BHT) in conditions of low temperature stress and high salinity (3, 0 MNaCl), irradiated by acute doses of UV-B light: 1 – control; 2 -; 25 mkM 2,6 di-tret-butyl cresol; 3 - 50 mkM 2,6 di-tret-butyl cresol

In figure 3 have been presented the results of photosynthetic oxygen evolution irradiated by various acute doses of UV-B light in control Dunaliella cells and the cells modified within 24 hours at intensive cultivation with concentrations 25 and 50 mkM of 2,6 di-tret-butyl cresol (BHT) in conditions of low temperature stress and high salinity. As seen in the figure, in control cells, irradiated by acute doses 15* 10^3 Erg\ mm^2, functional activity is suppressed 94%. The following increase of acute UV-B irradiation doses up to 18* 10^3 Erg\ mm^2 significantly reduces cell functioning to 74% (fig.3.curve.1.). The Acute dose 21* 10^3 Erg\ mm^2 leads to deeper suppression (35%) of the cell (photosynthetic oxygen evolution) function (fig.3.curve.1.). The cells modified by 2,6 di-tret-butyl cresol at concentration 25 mkM under the influence of acute doses of UV-B irradiation 15* 10^3 Erg\ mm^2, do not show high functional stability 86% compared to control cells. The increase of acute UV-B irradiation doses up to 18* 10^3 Erg\ mm^2 suppresses functional activity of the modified cells 74%. But acute UV-B irradiation doses 21* 10^3 Erg\ mm^2 significantly reduces (37%) photosynthetic oxygen evolution of cells modified by BHT (fig.3.curve.2.). The increase of concentration (50mkM) of synthetic antioxidant ionol in cell modification, has been shown that, stability of functional activity remains on high level 99%, under acute UV-B irradiation doses 15* 10^3 Erg\ mm^2. Acute doses 18* 10^3 Erg\ mm^2 of UV-B irradiation suppress functional activity of cells (80%), which differs from control cells, where suppression is 74%. The increase of acute UV-B irradiation doses up to 21* 10^3 Erg\ mm^2 reduces functional activity of cells till 56%. These are high indications of stability of functional activity of cells modified by BHT in conditions of low temperature stress and high salinity compared to control cells (35%).

The investigation carried out with antioxidant 2,6 di-tret-butyl phenol has showed that, this synthetic antioxidant demonstrates a tread role under the UV-B light influence.



Temperature 40%, light intensity 100 Wt\m^2

Fig 4: Photosynthetic oxygen evolution of control Dunaliella cells and the cells grown in medium with various concentrations of 2,6 di-tret-butyl phenol in conditions of low temperature stress and high salinity (3,0 MNaCl), irradiated by acute doses of UV-B light: 1 – control; 2 - 25 mkM 2,6 di-tret-butyl phenol; 3 - 50 mkM 2,6 di-tret-butyl phenol

In figure 3 have been presented the results of photosynthetic oxygen evolution irradiated by various acute doses of UV-B light in control Dunaliella cells and the cells modified within 24 hours at intensive cultivation with concentrations 25 and 50 mkM of 2,6 di-tret-butyl phenol in conditions of low temperature stress and high salinity. As seen in the figure, in control cells, irradiated by acute doses on range 15* 10^3 Erg\ mm^2 - 21* 10^3 Erg\ mm^2, cell function is strongly suppressed (fig.4.curve.1.). The modification of cells by 2,6 di-tret-butyl phenol in concentration 25mkM has showed that, this synthetic antioxidant demonstrates weak tread functioning of functional activity of cells against acute doses UV-B irradiation (fig.4.curve.2.). So, at acute dose 15* 10^3 Erg\ mm^2 of UV-B irradiation, the protection of functional cell activity is 65% compared to control cells 57%, and at acute dose 18* 10^3 Erg\ mm^2, the functional stability (49%) slightly differs from control cells (47%) (fig.4.curve.2.). The acute dose 21* 10^3 Erg\ mm^2 suppresses function of cells modified by 2, 6 di-tret-butyl phenol lower compared to control cells. The increase of antioxidant concentration up to 50mkM markedly has raised the tread function of 2, 6 di-tret-butyl phenol. Modification of cells at this concentration noticeably increses functional cell stability where s is observed smooth decrease of functional activity under the increase of UV-B irradiation. (fig.4.curve.3.). Thus, the functional protection of cells by synthetic antioxidants by 2, 6 di-tret-butyl cresol and 2, 6 di-tret-butyl phenol at low concentrations protect the functional activity of cells unequally. Probably, protective function of 2, 6 di-tret-butyl cresol (BHT – classic synthetic antioxidant) increases the tread function of analogue 2, 6 di-tret-butyl phenol.

4. Conclusions

1. It has been shown that, synthetic antioxidants by 2,6 di-tret-butyl cresol and 2,6 di-tret-butyl phenol in mineral medium, in conditions of low temperature stress and high salinity stimulate growth (1,5-8%) of population of Dunaliella cells compared to control.
2. Population of Dunaliella algae modified by synthetic antioxidants in conditions of low temperature stress and high salinity manifests higher functional stability against various acute UV-B irradiation doses compared to control cells.

5. References

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