Biotechnology of increasing the productivity of plants under the influence of microbiological preparations

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Abstract: Scientific and research work was carried out to determine the bioregulatory properties of Botryococcus, Chlorococcus, Tetradesmus Scenedesmus strains in plant growth. In the initial stages of the research, the influence of these microwater strains on the general germination of the seeds of the medium-ripe cotton variety "Bukhara-10" was studied, in which the international gene bank stored in the scientific laboratory "Biotechnology" of the Tashkent Institute of Chemical Technology was studied. Registered microalgae were used as main objects in our research. Key words: *Botryococcus, Chlorococcum, Tetradesmus, Chlorella, B.braunii* - AnDI-115, *Tetradesmus obliquus*-AnDI-015, *Scenedesmus quadricauda*- AnDI-44, *Chlorococcum infusionum* – AnDI -76, cotton.

1 Introduction

Due to the sharp increase in the demand for food and nutrients in the world, the increasing pressure of natural and anthropogenic influences on water and land resources, the loss of biodiversity and the sharp reduction of natural resources are causing serious problems in the development of sustainable agriculture and in providing humanity with agricultural products at the level of demand during the global climate change [1-13]. In the conditions of global climate change, it is necessary to effectively use innovative biotechnological facilities as alternative sources for the production of renewable energy sources and to ensure food security through the development of green biotechnologies and sustainable agriculture [14-24].

2 Results and Analysis

In the research work, in the scientific laboratory of the "Biotechnology" department of the Tashkent Institute of Chemical Technology, selection works were carried out to evaluate the effect of microalgae isolated from different regions of the country and presented for research on the germination of cotton seeds (Table - 1).

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Table-1. contains information about microalgae stored in the Tashkent Institute of Chemical Technology, "Biotechnology" scientific laboratory and registered in the NCBI international gene bank, these objects were used as the main objects in our research.

Table 1. Information about microalgae used in research

№	Name of the source used	Resource allocation	International Gene Bank Accession Number	The author	Source
1	*Botryococcus braunii*-AnDI-115	Izboskan district, Andijan region, Uzbekistan	MZ148226	Sohibov B.O.	https://www.ncbi.nlm.nih.gov/nuccore/MZ148225
2	*Tetradesmus obliquus*-AnDI-015		MZ148228	Sohibov B.O.	https://www.ncbi.nlm.nih.gov/nuccore/MZ148228
3	*Scenedesmus quadricauda*- AnDI-44		MZ148229	Sohibov B.O.	https://www.ncbi.nlm.nih.gov/nuccore/MZ148229
4	*Chlorococcum infusionum* – AnDI -76		MZ148226	Sohibov B.O.	https://www.ncbi.nlm.nih.gov/nuccore/MZ148226
5	*Chlorococcum humicola*-AnDU- 03	Ulugnor district, Andijan region, Uzbekistan	MZ148227	Sohibov B.O.	https://www.ncbi.nlm.nih.gov/nuccore/MZ148227

It is known from scientific sources that the objects of microalgae listed in Table – 1 are widely used in many sectors of the economy, including pharmaceuticals for obtaining biologically active substances, vitamins, emollient and toning gels for healing wounds, and preparations with antioxidant properties, in the food industry It is widely used as protein-vitamin-rich bioactive supplements, lipid-preserving agents at a high level, as means of protein-vitamin-lipid enrichment of children's food. Also, in recent years, it is widely used as the main sources for obtaining bioethanol and biodiesel, which is one of the most urgent issues. In addition, it is widely used for the production of nutritious feed products for the poultry and fishery industry and as live feed.[1]

Despite the wide range of applications of these microalgae, there is insufficient information in scientific sources about the importance and features of microalgae belonging to the genera Botryococcus, Chlorococcum, Tetradesmus in the management of plant growth in agriculture. Extensive information is provided in scientific sources about the properties of strains belonging to the genera of these microalgae, mainly in oil extraction, lipid or biodiesel production.[1]

It is appropriate to note that there are several scientific sources on the use of biomass or culture fluid of microalgae belonging to the genus Scenedesmus listed in Table 1 as a biostimulant in the cultivation of agricultural crops.[1]

Extensive information is provided in scientific sources about the properties of strains belonging to the genera of these microalgae, mainly in oil extraction, lipid or biodiesel production. Generally, in agriculture, there are extensive scientific sources on the control of plant growth and biological activity of microalgae belonging to the genera Chlorella and Chlamydomonas against phytopathogenic microorganisms.[1]

Therefore, in our research work, the bioregulatory nature of the strains belonging to the genus Botryococcus, Chlorococcum, Tetradesmus Scenedesmus, whose production processes are easy to organize, and whose growth is somewhat faster than that of microalgae belonging to the genus Chlorella research work was carried out to determine its characteristics. In the initial stages of the research, the effect of these microalgae strains on

the total fertility of the seeds of mid-season Bukhara-10 varieties of cotton was determined (Table - 2).

Table 2. Results of selection of collected microalgae strains on seed germination in laboratory conditions

№	Tajriba namunalari	Average fertility indicators of seeds, %					
		Observation in days					
		3	5	7	10	12	15
1	*Botryococcus braunii - AnDI-115*	-	23,23±1,12	48,42±0,19	86,41±0,36	100	
2	*Chlorococcum infusionum – AnDI -76*	-	18,02±1,43	31,61±0,24	76,37±1,16	96,41±0,18	100
3	*Chlorococcum humicola - AnDU- 03*	-	19,17±0,06	29,12±0,13	71,13±0,98	88,41±1,33	100
4	*Tetradesmus obliquus - AnDI-015*	-	11,34±0,17	42,11±0,27	61,27±0,12	78,23±1,17	100
5	*Scenedesmus quadricauda -AnDI-44*	-	10,42±0,34	27,14±0,08	61,51±0,27	83,36±0,21	100
6	*Control (water)*	-	3,98±0,08	12,71±0,13	57,41±1,32	79,54±0,08	90,62±0,12
7	*Control* (nutrient medium in which microalgae are grown)		6,81±1,11	21,12±1,36	69,41±1,17	82,14±0,37	96,14±0,24

Note - although there are signs of swelling, they are not obvious; - Microalgae cultures are grown in traditional, standard Tamiya nutrient medium; The number of seeds in each sample is 30 pieces, each option is repeated 5 times. Wet camera method was used for seed processing. Light - 2500 Lk; EKF-≤ 0.01 In research, in order to study the effect of microalgae on germination, seed germination was carried out in pots with a height of 40 cm in sandy conditions.

When analyzing the data presented in Table -2, two options were used as a control, the reason for this was the nutrient medium for the culture of sterile cultured microalgae and clean sterile water. When we analyzed the obtained results, it was observed that the biological activity of B. braunii - AnDI-115 strain, belonging to the genus Botryococcus, is somewhat high. In particular, when we compared the effect of the B. braunii-AnDI-115 strain on seed germination compared to the control variant, it was noted that 83% of all seeds germinated on the 5th day of observation. On the basis of the observations made on the same day, it was noted that the germination of seeds in the culture liquid of the 7th variant was 6.81%, and compared to the B. braunii-AnDI-115 strain, it showed 71% less biological activity.

Also, when comparing the biological activity of B.braunii-AnDI-115 strain to representatives of the genus Chlorococcum, on the 5th day of observation, Chlorococcum infusionum - AnDI-76 strain - 18.02%, and Chlorococcum humicola - AnDU-03 strain - 19.17% biological activity showing activity, it was found that 78% higher than control (water) in variant 6, and 63% higher than control in variant 7. When we compared the biological activity of the strains belonging to the Chlorococcum genus to the B. braunii-AnDI-115 strain, it was observed that the Chlorococcum infusionum-AnDI-76 strain - 23%, and the Chlorococcum humicola-AnDU-03 strain showed 18% less viability. Also, although Chlorococcum infusionum-AnDI-76 and Chlorococcum humicola-AnDU-03 strains did not show significant inter-strain differences on the 5th day of observation, by the 7th day of observation, inter-strain biological activity differed up to 8.0%. It was noted that the differences in the same range remained until the last day of observation.

During the research, it was found that by the seventh day of observation, B. braunii-AnDI-115 strain showed 73.8% higher efficiency compared to the control in option 6 (water), and 56.4% compared to the control in option 7 (Tamiya nutrient medium). At this point, when we compared the control options, it was noted that the seed germination rate was 3.98% in plain water on the 5th day of observation, while the seed germination rate

was 6.81% in the control liquid of the 7 th option. When both controls were compared, it was noted that the nutrient medium in which microalgae was grown showed 41.6% higher biological activity compared to pure water.

It was concluded that this high biological efficiency may have been caused by the chemical elements used to grow microalgae in the nutrient medium. Based on a comparative comparison of the data presented in Table -2, the Tetradesmus obliquus-AnDI-015 strain showed a higher rate of 65.0% on the 5th day of observation compared to the control option in option 6, and compared to the control option in option 7. It was noted that 40.0% showed a high rate. Scenedesmus quadricauda-AnDI-44 strain was noted to be 61.9-34.7% more effective than the control variants on the 5th day of observation. On the 10th day of observation, the B. braunii-AnDI-115 strain belonging to the genus Botryococcus provided 86.41% germination, while the Chlorococcum infusionum-AnDI-76 strain showed 76.13% germination, which was superior to the B. braunii-AnDI-115 strain. showed 11.7% low performance, while Chlorococcum humicola-AnDU-03 strain showed 71.13% germination and showed 17.7% low performance compared to B.braunii-AnDI-115 strain done.

Also, Scenedesmus quadricauda-AnDI-44 and Tetradesmus obliquus-AnDI-015 strains showed very close values, being 28.9-29.1% lower than B. braunii-AnDI-115 strain, respectively. it was noted that the indicator showed. It was noted that the B. braunii-AnDI-115 strain showed a 33.6% higher rate compared to the control in the 6th option, and 19.7% compared to the control in the 7th option, on the 10th day of observation.

It is known from scientific data that 46 species of blue-green algae (Cyanophyta) and green algae (Chlorophyta) synthesize indole acetic acid, including indolyl-3-butyric acid (IMK), indolyl-3 -propionic acid (IPK) and indolyl-3-acetamide (IAM) synthesis [2].

Also, it is noted in many scientific sources that microalgae store auxin-like substances in the culture medium, that is, in the culture liquid, in the biomass of cells, and in most cases in the extract mass obtained from the biomass.[1]

In the results presented in Table -2, it was noted that all microalgae strains used in the study had a positive effect on seed germination compared to the control. However, based on the results, it is necessary to scientifically substantiate that B.braunii-AnDI-115 strain and Chlorococcum infusionum-AnDI-76 strain recorded higher results compared to other microalgae.

Therefore, we tried to study the differences in chlorophyll storage and IUK synthesis of these research objects as the main factors affecting seed germination. The obtained results may be the basis for the differences in permeability between microalgae.

As we know, the dark green color of microalgae is one of the factors determining its biological activity and moderate growth. Therefore, in preliminary studies, the differences of the objects shown in Table -1 according to the storage of chlorophyll-a, chlorophyll-b and total chlorophyll were studied. The obtained results are shown in Table -3.

On the basis of the research conducted in order to study the effect of the microalgae on seed germination, their dependence on chlorophyll storage properties.

When the obtained results were analyzed, the total pigment production index of Botryococcus braunii - AnDI-115 strain, which showed 86.41% result on the 10th day of observation of seed germination, was 0.29 mg/l, 0.81 mg/l, 1.32 mg/l, 3.12 mg/l, and its average chlorophyll content during the growth phase was 1.39 mg/l.

Table 3. Analysis of pigment formation properties of microalgae

№	Samples	Duration of cultivation, in days				Daily average total chlorophyll content, mg/l
		3	5	7	10	
		Total chlorophyll storage, mg/l				
1	*Botryococcus braunii - AnDI-115*	0,29±0,03	0,81±0,23	1,32±0,08	3,12±1,04	1,39±0,35

2	*Chlorococcum infusionum-AnDI-76*	0,31±0,18	0,92±1,07	1,56±1,06	3,91±0,33	1,68±0,42
3	*Chlorococcum humicola - AnDU- 03*	0,09±0,06	0,36±0,16	0,89±0,03	2,11±0,17	0,81±0,11
4	*Tetradesmus obliquus - AnDI-015*	0,11±0,16	0,31±0,13	0,87±0,27	1,81±0,06	0,78±0,14
5	*Scenedesmus quadricauda - AnDI-44*	0,19±0,27	0,78±0,22	1,16±0,19	2,36±1,12	1,12±0,42

Note: EKF with confidence interval ≤ 0.01

3 Discussion

Chlorococcum infusionum - AnDI -76 strain provided 76.37% seed germination on the 10th day of observation, while the chlorophyll retention index was 0.31 mg/l, 0.92 mg/l, respectively, by day. , 1.56mg/l, 3.91mg/l, and it was found that the average chlorophyll content in the growth phase was 1.68 mg/l. So, it can be seen that the results presented in Table-2 are not confirmed by the data recorded in Table-3

This condition can also be observed in other microalgae cultures. In particular, it was observed that Chlorococcum humicola-AnDU-03 strain and Chlorococcum infusionum-AnDI-76 strain showed different indicators. However, it can be seen that the strains belonging to the genus Chlorococcum did not significantly differ from each other in terms of the indicators of ensuring the germination of the seed. However, Chlorococcum infusionum-AnDI-76 strain 0.31 mg/l on the 3rd day of cultivation, 0.92mg/l on the 5th day, 1.56mg/l on the 7th day, 1.56mg/l on the 10th day - 3.91 mg/l of pigment was produced, while during the growth phase it was noted that it produced an average of 1.68 mg/l of chlorophyll.

Chlorococcum humicola-AnDU-03 strain kept 0.09mg/l, 5th day 0.36mg/l, 7th day 0.89mg/l, 10th day 2.11mg/l chlorophyll, development during the phase, it can be seen that 0.81 mg/l of chlorophyll was stored. Even in this case, the general chlorophyll storage characteristic of cultures does not allow to consider microalgae as the main factor in ensuring seed germination.

During the research, although Scenedesmus quadricauda -AnDI-44 strain provided 61.51% seed germination on the 10th day of observation, an average of 1.12mg/l chlorophyll storage was observed according to the total chlorophyll storage. Also, Tetradesmus obliquus- AnDI Although the -015 strain also provided 61.27% seed germination on the 10th day of observation, it can be seen that it retained an average amount of 0.78mg/l chlorophyll during the development phase. These data also show that the total chlorophyll storage index of microalgae does not play a major role in ensuring seed germination.

It is known from scientific sources that in the large-scale scientific research conducted in recent years,[1] it is noted that chlorophyll a and chlorophyll b play an important role in the growth of plants, and it is chlorophyll b that is important.[1]

Also, the scientific conclusions that the high chlorophyll storage capacity of microalgae is one of the factors ensuring the perfect biochemical composition of microalgae have been noted in the works of many researchers.[1]

Based on the scientific analyzes conducted during our research, we considered the initial conclusions based on the data presented in Table -3 to be insufficiently scientifically based, and we came to the conclusion that it is not enough to determine the total chlorophyll content of the microalgae used as research objects.

Therefore, we tried to analyze the storage parameters of chlorophyll a and chlorophyll b of the studied microalgae and their correlation. The obtained results are shown in table -4. When analyzing the obtained results, according to the characteristics of the total pigment

formation of the studied microalgae, the Botryococcus braunii-AnDI-115 strain produces pigment in the amount of 23.08 mg/mln cells, while the amount of chlorophyll a compared to the total pigment is 12.45 mg/mln cells, it can be observed that the ratio of chlorophyll a and chlorophyll b is 1.75. It was noted that the amount of carotenoids is 15.22% compared to the amount of total pigments.

It was observed that the total amount of pigments of the strains belonging to the Chlorococcum genus was 17.56 mg/mln. cells in the Chlorococcum infusionum-AnDI-76 strain, and 20.44 mg/mln cells in the Chlorococcum humicola-AnDU-03 strain.

When comparing the amount of chlorophylls of both strains, the amount of chlorophyll a was 9.26 mg/mln cells in Chlorococcum infusionum-AnDI-76 strain, and 10.07 mg/mln cells in Chlorococcum humicola-AnDU-03 strain, chlorophyll The ratio of a and chlorophyll b was found to be 1.65 and 1.41, respectively. It was noted that the amount of carotenoids was from 15.20 percent to 15.71 percent, respectively, in relation to the total pigments of these strains.

Table 4. Dynamics of chlorophyll storage of microalgae in Tamiya nutrient medium (In the case of 15-day biomass, 3500 Lk)

№	Samples	Chlorophyll a storage, mg/mln cells					
		Growing in days					
		Chlorophyll *a*, mg/mln cells	Chlorophyll *b*, mg/mln cells	Amount of total carotenoids, %	Amount of total pigments	The amount of carotenoids relative to total pigments, %	Ratio of chlorophyll *a* and *b*
1	*Botryococcus braunii - AnDI-115*	12,45±0,12	7,11±0,08	3,52±0,23	23,08±0,17	15,22±0,16	1,75±0,23
2	*Chlorococcum infusionum – AnDI -76*	9,26±0,11	5,62±0,17	2,68±0,28	17,56±0,33	15,20±0,26	1,65±0,17
3	*Chlorococcum humicola - AnDU- 03*	10,07±0,27	7,16±0,13	3,21±0,08	20,44±0,21	15,71±0,33	1,41±0,18
4	*Tetradesmus obliquus - AnDI-015*	10,17±0,33	7,48±0,03	2,72±0,17	20,37±0,08	13,31±0,41	1,36±0,37
5	*Scenedesmus quadricauda - AnDI-44*	11,08±0,16	7,12±0,42	3,27±0,31	21,47±0,03	15,21±0,16	1,56±0,06

Also, the total pigment production of Tetradesmus obliquus-AnDI-015 strain is 20.37 mg/mln cells, in which the amount of chlorophyll a is 10.17 mg/mln. cells, the amount of chlorophyll b is 7.48 mg/mln cells, it was noted that the ratio of chlorophyll a and chlorophyll b was 1.36. It was observed that the ability of this strain to synthesize carotenoids is 2.72 mg/mln cells and is 13.31% compared to total pigments. The production of total pigments of Scenedesmus quadricauda-AnDI-44 strain is 21.47 mg/mln cells, of which chlorophyll a is 11.08 mg/mln cells, chlorophyll b is 7.12 mg/mln cells, and the amount of carotenoids is 3.27 mg/mln cells. It was noted that it is a million cells. When comparing the results presented in Table -2 with respect to the seed germination of microalgae, on the 12th day of observation (Table-2) Botryococcus braunii-AnDI-115 strain showed a hundred percent indicator, it can be concluded that the Chlorococcum infusionum-AnDI-76 strain showed 96.41% fertility, that is, it showed a higher rate compared to other microalgae strains.

If we relate seed germination to the storage of carotenoids in relation to the general pigments of microalgae, although there are significant differences in the percentage of storage of specific carotenoids, it was not possible to compare these results with indicators of seed germination. For example, if Botryococcus braunii-AnDI-115 strain provided 100% seed germination on the 12th day, we can see that its carotenoid content is 15.22% compared to total pigments.

The same situation can be observed in the Scenedesmus quadricauda-AnDI-44 strain, i.e., the carotenoid content of total pigments was 15.21%. However, if we pay attention to the effect of this strain on seed germination, it showed 83.36% germination on the 12th day of observation. The same situation can be observed in Tetradesmus obliquus - AnDI-015 strain, i.e., its total pigment production was 20.37 mg/mln. cells, and the amount of carotenoids in relation to total pigments was 13.31%.

If we pay attention to the seed germination of this strain, it can be seen that it showed 78.23% germination on the 12th day of observation. Therefore, the effect of cultures on seed germination cannot be related to the percentage of carotenoids in relation to total pigments. During the comparative study of these indicators, it was concluded that the main role may have been played by the ratio of chlorophylls a and b. For example, in Botryococcus braunii-AnDI-115 strain, the ratio of chlorophyll a and chlorophyll b was 1.75, and the activity of the same strain in seed germination was also noted. According to the same indicator, including Chlorococcum infusionum-AnDI-76 strain showing 96.41% germination, Chlorococcum humicola - AnDU- 03, Tetradesmus obliquus - AnDI-015 and Scenedesmus quadricauda -AnDI-44 strains in a greater proportion, that is, it can be noted that it synthesizes chlorophyll a and chlorophyll b in a ratio of 1.65.

Also, it was observed that the Scenedesmus quadricauda-AnDI-44 strain provided 83.36% seed germination and showed a 5.13% higher rate than the Tetradesmus obliquus-AnDI-015 strain, while this strain with a ratio of chlorophylls (1.56) synthesizes a significant amount of chlorophyll to compare with the strain of Tetradesmus obliquus-AnDI-015 (1.36 ratio). Thus, when analyzing the results of the conducted research, it was proved that the ratio of chlorophyll a and chlorophyll b can be considered as a factor that plays a key role in controlling the effect of microalgae on seed germination.

The obtained results were compared with respect to the total fertility and the periods of maximum fertility. It was observed that the B. braunii-AnDI-115 strain gave a hundred percent result on the 12th day of observation, while the Chlorococcum infusionum-AnDI-76 strain was 96.41%. Based on the requirements for the general fertility of seeds of agricultural crops, B.braunii-AnDI-115 strain belonging to the Botryococcus genus and Ch.infusionum-AnDI-76 strain belonging to the Chlorococcum genus were selected for further research.

It is known from scientific sources that one of the main factors determining the economic efficiency in the process of preparing biological fertilizers based on microalgae and cyanobacteria is the cost of the nutrient medium during their cultivation, methods of cultivation and forms of application. [4]

4 Conclusion

Microalgae registered in the international gene bank Botryococcus braunii-AnDI-115, Tetradesmus obliquus-AnDI-015, Scenedesmus quadricauda -AnDI-44, Chlorococcum infusionum - AnDI-76 and Chlorococcum humicola-AnDU-03, based on the Bukhara - 10 medium-sized variety screening was conducted on the effect of cotton on seed germination. According to the results of the research, it was noted that the studied cultures differ according to the content of chlorophyll-a, chlorophyll-b and total chlorophyll, it was

determined that the ratio of chlorophyll a and chlorophyll b should be considered as a factor that plays a key role in controlling the effect of microalgae on seed germination.

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