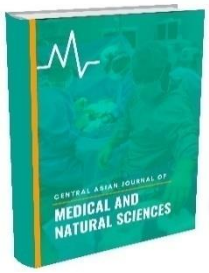
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 03 | May- June 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Evaluation of the Effect of Microbiological Biopreparations On Seed Germination

Muhayo Bafoevna Tagaeva
Tokhirov Bakhtiyor Bakhshullaevich

Received 22nd Mar 2023,
Accepted 23rd Apr 2023,
Online 12th May 2023

Bukhara State University

Abstract: In the article *Chlorella* compared to the microalgae belonging to the generation, which is carried out somewhat quickly *Botryococcus, Chlorococcum, Tetradesmus Scenedesmus* detailed information on the identification of bioregulatory properties of strains belonging to the genus in the growth of plants.

Key words: *Botryococcus, Chlorococcum, Tetradesmus, Scenedesmus,*microalgae *Botryococcus brownie*-AnDI-115, *Tetradesmus obliquus*-AnDI-015, Scenedesmus quadricauda-AnDI-44, *Chlorococcum infusionum* –AnDI-76, *Chlorococcum humicola*-AnDU-03

In the research works, selection works were carried out in the scientific laboratory of the Department of Biotechnology of the Tashkent Institute of Chemical Technology to assess the effect of microalgae isolated from different regions of the country and presented for research on cotton seed germination (Table 3.1.1). Table 3.1.1 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 object in our research.

Table 3.1.1

Information about microalgae used in research

No	Name of the source used	Resource allocation	NCBI International Gene Bank Accession Number	The author	Source
1	*Botryococcusbrownie*-AnDI-115	Uzbekistan, Andijan region,	MZ148226	Sahibov BO	https://www.ncbi.nlm.nih.gov/nuccore/MZ148225

2	*Tetradesmus obliquus*-AnDI-015	Izboskan district	MZ148228	Sahibov BO	https://www.ncbi.nlm.nih.gov/nuccore/MZ148228
3	*Scenedesmus quadricauda* - AnDI-44		MZ148229	Sahibov BO	https://www.ncbi.nlm.nih.gov/nuccore/MZ148229
4	*Chlorococcum infusionum* – AnDI-76		MZ148226	Sahibov BO	https://www.ncbi.nlm.nih.gov/nuccore/MZ148226
5	*Chlorococcum humicola*-AnDU-03	Uzbekistan, Andijan region, Ulughnor district	MZ148227	Sahibov BO	https://www.ncbi.nlm.nih.gov/nuccore/MZ148227

It is known from scientific sources that the objects of microalgae listed in Table 3.1.1 are widely used in many sectors of the economy, including in pharmaceuticals for the production of biologically active substances, vitamins, emollient and toning gels for wound healing, and preparations with antioxidant properties, in the food industry, protein-vitamin-rich bioactive additives, high As lipid-preserving agents, balls are widely used as means of protein-vitamin-lipid enrichment of food products. Also, it is widely used as the main sources in the production of bioethanol and biodiesel, which is one of the most pressing issues in recent years. In addition, it is widely used for the production of nutritious feed products for the poultry and fishery industry and as live feed [].

Despite the fact that the fields of application of these microalgae are very wide, precisely *Botryococcus, Chlorococcum,Tetradesmus* There is insufficient information in scientific sources about the importance and characteristics of microalgae belonging to the genus in the management of plant growth in agriculture. Extensive information on the properties of strains belonging to this microalgae genera, mainly in oil extraction, lipid or biodiesel production [].

It is appropriate to note that there are several scientific sources on the use of microalgae belonging to the genus Scenedesmus in table 3.1.1 as a biostimulant in the cultivation of agricultural crops [].

Extensive information on the properties of strains belonging to this microalgae genera, mainly in oil extraction, lipid or biodiesel production, is covered in scientific sources. In general, there are extensive scientific resources on the control of plant growth and biological activity of microalgae belonging to the genera Chlorella and Chlamydomonas in agriculture [Gitau et al., 2021].

Therefore, it is easy to organize production processes in our research work, to grow and develop *Chlorella* compared to the microalgae belonging to the generation, which is carried out somewhat quickly *Botryococcus, Chlorococcum, Tetradesmus Scenedesmus* Research works were carried out to determine the bioregulatory properties of the strains belonging to the genus in the growth of plants. In the initial stages of the research, the effect of these microalgae strains on the total fertility of early Bukhara-108 cotton seeds was determined (Table 3.1.2). In research, in order to study the effect of micronutrients on germination, germination of seeds was carried out in pots with a height of 40 cm in sandy conditions.

Table 3.1.2

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

No	Examples of experience	Average fertility indicators of seeds, % observation days					
		3	5	7	10	12	15
1	*Botryococcusbraunii - 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*(microalgae culture medium**)		6.81±1.11	21.12±1.36	69.41±1.17	82.14±0.37	96.14±0.24

Explanation– *- although there are signs of unsoundness, they are not obvious; **-Microalgae cultures were grown in traditional, standard Tamiya nutrient medium; The number of seeds in each sample was 30, each variant was repeated 5 times, and the wet chamber method was used for seed processing. Light – 2500 Lk; EKF-≤ 0.01

When we analyzed the data presented in Table 3.1.2, two options were used as controls, namely sterile cultured microalgae culture medium and pure 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 B.braunii-AnDI-115 strain on seed germination compared to the control option, it was noted that 23.23% 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, the biological activity of B. braunii-AnDI-115 strain *Chlorococcum*on the 5th day of observation, Chlorococcum infusionum - AnDI-76 strain - 18.02%, and Chlorococcum humicola - AnDU-03 strain showed 19.17% biological activity, compared to the control (water) in the 6th option, 78%. Compared to the control in the 7th option, it was found that 63% showed a higher indicator. Biological activity of strains belonging to the representatives of the genus Chlorococcum*B. braunii-*

Compared to strain AnDI-115, *Chlorococcum infusionum*-It was observed that strain AnDI-76 - 23%, and strain Chlorococcum humicola-AnDU-03 showed 18% low fertility. 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%. Differences in the same range were observed until the last day of observation.

During the research*B. Braunii* By the seventh day of observation, the 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 germination of seeds in normal water was 3.98% on the 5th day of observation, while the germination of seeds in the control liquid of the 7th option was 6.81%. When both controls were compared, it was noted that the microalgae nutrient medium showed 41.6% higher biological activity than pure water.

It was concluded that this high biological efficiency may have been caused by the chemical elements used for the cultivation of microalgae in the nutrient medium. Based on the comparative comparison of the data presented in Table 3.1.2, the Tetradesmus obliquus-AnDI-015 strain showed a 65.0% higher rate compared to the control variant 6 on the 5th day of observation, and a 40.0% higher rate compared to the control variant 7 it was noted that it showed. 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 *Botryococcus* belongs to the generation*B. braunii*-AnDI-115 strain provided 86.41% fertility,*Chlorococcum infusionum*-AnDI-76 strain showed 76.13% viability,*B. braunii*- 11.7% lower performance compared to AnDI-115 strain, *Chlorococcum humicola*-And the AnDU-03 strain showed 71.13% viability, *B. braunii*17.7% lower than AnDI-115 strain was noted.

Also, it was noted that Scenedesmus quadricauda-AnDI-44 and Tetradesmus obliquus-AnDI-015 strains showed very close indicators compared to each other and showed a 28.9-29.1% lower indicator compared to B.braunii-AnDI-115 strain, respectively. . 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 thatsynthesis of indole acetic acid in 46 species of blue-green algae (Cyanophyta) and green algae (Chlorophyta), including indolyl-3-butyric acid (IMC), indolyl-3-propionic acid (IPK) and indolyl-3-acetamide (IAM) is noted to synthesize [Romanenko E.A., 2015].

It is also 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 [Shanab et al., 2003; Plettner et al., 2005; Varalakshmi et al., 2012].

In the results presented in Table 3.1.2, 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, B. braunii-AnDI-115 strain and *Chlorococcum infusionum-AnDI-76*Scientific justification is required that the strain recorded higher results compared to other microalgae.

Therefore, we tried to study the differences in their chlorophyll storage and IUK synthesis as the main factors affecting the seed germination of these research objects. The obtained results may be the basis for differences in viability 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 3.1.1 according to chlorophyll-a, chlorophyll-b and total chlorophyll storage were studied. The obtained results are shown in table 3.1.3.

Table 3.1.3

Analysis of pigment formation properties of microalgae

No	Samples	Cultivation duration, days				Daily average total chlorophyll content, mg/l
		3	5	7	10	
		Total chlorophyll content, mg/l				
1	*Botryococcusbraunii - 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: confidence interval EKF ≤ 0.01

On the basis of the studies carried out in order to study that the studied microalgae showed different indicators on the effect on seed germination, their dependence on chlorophyll storage properties

When the obtained results were analyzed, Botryococcus showed 86.41% results on the 10th day of observation of seed germination. *brownie*- If the total pigment formation index of strain AnDI-115 was 0.29 mg/l, 0.81 mg/l, 1.32 mg/l, 3.12 mg/l during the observed days of cultivation, its growth - it was noted that the average chlorophyll content during the development phase was 1.39 mg/l. 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, 1.56 mg/l, and 3.91 mg/l, respectively. and it was found that the average chlorophyll content in the growth phase was 1.68 mg/l. Therefore, it can be seen that the results presented in Table 3.1.2 are not confirmed by the data recorded in Table 3.1.3.

This situation 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 were not significantly different 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, 3.91mg on the 10th day /l produced pigment, 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 on the 3rd day of cultivation, 0.36mg/l on the 5th day, 0.89mg/l on the 7th day, 2.11mg/l on the 10th day, and 0. It can be seen that 81mg/l of

chlorophyll is preserved. 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 *Scenedesmus quadricauda* -While strain AnDI-44 provided 61.51% seed germination on the 10th day of observation, an average of 1.12mg/l chlorophyll retention was observed in terms of total chlorophyll retention, and also strain Tetradesmus obliquus- AnDI-015 seed germination on day 10 of observation was 61.27 %, it can be seen that during the development phase, the average amount of chlorophyll was 0.78mg/l. These data also show that the total chlorophyll content of microalgae does not play a major role in ensuring seed germination.

It is known from scientific sources that chlorophyll a and chlorophyll b play an important role in plant growth in large-scale scientific research conducted in recent years [Espineda et al., 1999; Beale et al., 1999], in which it is noted that chlorophyll b plays an important role [Nakagawara et al., 2007; Sakuraba et al., 2010].

Also, the scientific conclusions that the high chlorophyll storage capacity of microalgae is one of the factors that ensure the perfect biochemical composition of microalgae have been noted in the works of many researchers [Elizarova, 1974, 1975; Dere et al., 1998].

Based on the scientific analyzes carried out during our research, considering the initial conclusions based on the data presented in Table 3.1.3 as insufficiently scientifically based, 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 indicators of chlorophyll a and chlorophyll b storage of the studied microalgae and their correlation. The obtained results are shown in table 3.1.4. When we analyze the obtained results, according to the general pigment-forming properties of the studied microalgae *Botryococcusbrownie*-If the AnDI-115 strain produces pigment in the amount of 23.08 mg/mln. cells, it can be observed that the amount of chlorophyll a is 12.45 mg/mln. cells compared to the total pigment, and 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 total amount of pigments.

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

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 a and chlorophyll The ratio b was found to be 1.65 and 1.41, respectively. It was noted that the amount of carotenoids in relation to the total pigments of these strains was 15.20 to 15.71 percent, respectively.

Table 3.1.4

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

No	Samples	Chlorophyll a storage, mg/mln. cells					
		Cultivation days					
		Chlorophyll a, mg/mln.	Chlorophyll b, mg/mln.	Amount of total carotenoids, %	Amount of total pigments	Amount of carotenoids in relation to total pigments, %	ratio of chlorophyll a and b
1	*Botryococcusbraunii - 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*Tetradesmus obliquus*-The total pigment formation of 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, chlorophyll a and it was noted that the ratio of 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 the total pigments.

Scenedesmus quadricauda- The production of total pigments of AnDI-44 strain is 21.47 mg/mln. cells, in 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 was noted. When comparing the results presented in Table 3.2.2 with respect to the seed germination of microalgae, on the 12th day of observation (Table 3.1.2) *Botryococcus braunii-AnDI-115*strain showed a hundred percent indicator, it can be concluded that Chlorococcum infusionum-AnDI-76 strain showed 96.41% viability, that is, it showed a higher indicator compared to other microalgae strains, which may depend on the ratio of microalgae in keeping chlorophyll a and chlorophyll b.

In relation to seed germination, carotenoid storage versus total pigments of microalgae, although there were significant differences in specific carotenoid storage percentages, it was not possible to compare these results with seed germination parameters. For example, Botryococcus*braunii-AnDI-115*strain provided hundred percent seed germination on the 12th day, we can see that its carotenoid content is 15.22% compared to total pigments.

The same situation *Scenedesmus quadricauda* It can also be observed in the AnDI-44 strain, that is, the carotenoid content in relation to total pigments was 15.21%. However, if we focus on 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, that is, its total pigment production was 20.37 mg/mln. cells, and the amount of carotenoids in relation to total pigments was 13.31%.

Focusing on 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 cultivars on seed germination cannot be attributed to the percentage of carotenoids relative to total pigments. During the comparative study of these indicators, it was concluded that the ratio of chlorophylls a and b chlorophylls may have played a key role. Including *Botryococcusbraunii-AnDI-115*in the 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, compared to Chlorococcum humicola - AnDU-03, Tetradesmus obliquus - AnDI-015 and Scenedesmus quadricauda -AnDI-44 strains, i.e. chlorophyll a and It can be noted that it synthesizes chlorophyll in a ratio of 1.65.

Also *Scenedesmus quadricauda-AnDI-44*strainsowProviding 83.36% fecundity, it showed 5.13% higher rate compared to Tetradesmus obliquus-AnDI-015 strain, this strain also showed chlorophyll ratio (1.56) compared to Tetradesmus obliquus-AnDI-015 strain (1.36 ratio) It was observed that a considerable amount of chlorophyll was synthesized. 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 total fertility and periods of maximum fertility. If the B. braunii-AnDI-115 strain gave a hundred percent result on the 12th day of observation,*Chlorococcum infusionum*-It was observed that strain AnDI-76 was 96.41%. Botryococcus belongs to the genus Botryococcus for further research based on the requirements for the general fertility of agricultural crop seeds.*B. braunii*-AnDI-115 strain and*Chlorococcum*Ch.infusionum -AnDI-76 strains belonging to the genus were selected.

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