The influence of the chlorella suspension on morphometric parameters of different taxonomical groups plants seedlings

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Abstract. Microalgae chlorella contains a pool of biologically active substances, which creates the preconditions for its commercial production for use in agriculture, medicine, cosmetology and other areas of human activity. Specialized substances that affect seed germination, seedling growth and development, yield and decorative properties of plants for in vivo use are a wide variety. However, they can be toxic to humans and animals, as well as to the plants themselves. Biostimulants and biofertilizers based on macro- and microalgae have managed to establish themselves as an actual replacement for toxic substances, demonstrating high efficiency on plants of various taxonomic groups. The use of a biostimulant based on a suspension of chlorella in the study of seed germination and biometric parameters of seedlings showed the following results: leaf mass and volume, germination, stem and root length increased in plants of spruce, marigold, rye, barley, spelled, triticale, melissa, while in pine plants the germination decreased, and in cabbage plants it remained at the same level, which indicates a difference in the perception of chlorella suspension by plants of different taxonomic groups.

1 Introduction

Microalgae are microscopic single-celled plants that have high rates of growth and photosynthetic efficiency and relatively low natural resource requirements compared to traditionally grown crops.

They represent a huge biological diversity, of which about 40,000 objects have already been described or analyzed [1].

Now they are becoming more relevant due to the many advantages: food ingredients from them do not contain GMOs, they are organic and reduce the negative impact of the anthropogenic factor on the environment. For the production of products from them, there is no need for deforestation, so these technologies are in line with the current global trend for environmental sustainability. In addition, microalgae production has a low carbon, land and water footprint (i.e. low greenhouse gas emissions and land and water resource requirements).

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As for the methods of cultivating microalgae, these organisms require the main chemical elements for nutrition (nitrogen, oxygen, hydrogen, potassium, magnesium, sulfur, phosphorus, etc.), water and sunlight for the photosynthesis, however they do not need fertile soil to grow, because they are cultivated in suspension. Most often, systems of open (or semi-open) reservoirs or closed photobioreactors in the form of various installations are used [2].

Chlorella (*Chlorella* sp.) is a green eukaryotic microalgae. The microscopic cell is spherical, 2–10 µm in diameter. This microalgae is one of the most important and promising for biomass production [3].

Chlorella contains a pool of biologically active substances: about 50% protein (including essential amino acids); a complex of essential unsaturated fatty acids (including Omega-3); vitamins (A, B1, B2, B3, B5, B6, E) and macro- and microelements. This creates the prerequisites for its commercial production for use in agriculture, medicine, cosmetology and other fields.

Many known species of microalgae are quite demanding in terms of cultivation conditions: the level of illumination, the composition of the nutrient medium, the concentration of carbon dioxide, and mechanical mixing, which greatly hinders their effective cultivation. However, the green single-celled microalgae chlorella has been an object of biotechnology for quite a long period of time and is widely used as a vitamin and mineral supplement for farm animals, poultry and fish, as well as in the production of drugs in the chemical and pharmaceutical industries, which determines the choice of this microalgae as a source of valuable compounds for studying its effect on the biosynthetic potential and morphophysiological parameters of plants.

Specialized substances affecting seed germination, seedling growth and development, yield and decorative properties of plants for in vivo use represent a wide variety. However, they can be toxic to humans and animals, as well as to the plants themselves. The search for organic substances with a minimum of side effects and with availability, as well as relative cheapness, continues constantly. An increase in the morphophysiological parameters of higher plants in vivo due to such preparations, in turn, can increase the yield of economically important plants, which is valuable in the context of the agricultural significance of the development.

It has been shown that algae biomass can be used directly on crops as a foliar application or as a root application to accelerate plant development or increase overall yield. In addition, extracellular exudates of *Chlorella sorokiniana* in the culture medium increased the total dry biomass (aerial parts of the plant by 22% and underground by 51%) and the length of wheat plants (by 30%) compared with the control, which highlights the biostimulatory potential of extracellular metabolites from microalgae [4].

Biostimulants can also affect root development, which in turn affects the growth, health, and nutritional composition of the entire plant, as lateral roots improve water and nutrient uptake [5]. In addition, *Chlorella kessleri* is rich in phytohormones such as auxin and gibberellins, and when applied to *Vicia faba* in water extract improved germination, increased growth parameters, leaf area, pigment content, and sodium and potassium accumulation in roots and shoots compared to the control group [6]. Extracts of *Scenedesmus quadricauda* and *Chlorella vulgaris* have a biostimulating effect on the expression of genes associated with the absorption of nutrients by sugar beet *Beta vulgaris* [7].

One of the advantages of microalgae suspension as a fertilizer is that the microalgae biomass can be used directly without extraction of specific compounds when drying of the biomass is not required [8]. For example, *Chlorella* sp. was grown on wastewater, and wet biomass was applied directly to the soil. This caused the formation of a soil biofilm from microalgae, which enhanced the interaction of plants and microorganisms and led to an increase in the biostimulating effect compared to the control [9,10].

2 Methods

The object of the study was a strain of chlorella with a thick cell wall (*Chlorella vulgaris* Beijer), used as a component of a biostimulator for plants. The composition of the biostimulant includes: cultural aquatic medium, microalgae *Chlorella vulgaris*.

The biological activity of the chlorella suspension was tested on the seeds of *Picea rubra*, *Pinus sylvestris*, *Tagetes erecta*, *Brassica oleracea*, *Secale cereale*, *Hordeum vulgare*, *Triticum dicoccon*, ×*Triticosecale*, *Melissa officinalis*. Seeds were germinated on a layer of cotton wool moistened with 70 ml of chlorella suspension (experimental variant) or water (control). Seeds were germinated in plastic containers with a volume of 250 ml in a light room, where the temperature was maintained at 23°C, a 16-hour photoperiod, and illumination with white fluorescent lamps. The results were taken on the 30th day from the moment of sowing the seeds.

The studies were carried out in 9 biological and 2 analytical replicates. Averages of all data were calculated using Microsoft Excel 2013 (Microsoft Corporation, USA). Analysis of variance (ANOVA) was performed using Statistica version 10.0 and means were compared using Fisher's least significant difference (LSD) test at a $p \leq 0.05$ significance level.

3 Results

The results of the study of the different taxonomic groups plants seeds germination using a suspension of chlorella and distilled water allowed us to note the difference in the biometric parameters of seedlings (Table 1).

Table 1. Influence of chlorella suspension and distilled water on the germination and the biometric parameters of seedlings.

Plant	Chlorella suspension			Distilled water		
	Germination, %	**Stem length, см**	**Root length, см**	**Germination, %**	**Stem length, см**	**Root length, sm**
Spruce	76.7±3.9	2.3±0.1	0.9±0.05	36.7±1.8	2.0±0.1	1.6±0.1
Pine	43.3±2.2	1.9±0.1	0.9±0.05	63.3±3.2	2.8±0.1	1.0±0.1
Marigold	73.4±3.7	2.0±0.1	0.6±0.03	55.0±2.8	1.8±0.1	0.5±0.03
Cabbage	46.7±2.3	0.7±0.04	0.5±0.03	46.7±2.3	0.6±0.03	0.4±0.02

When using a suspension of chlorella, the seedlings took on a deformed appearance, and turned yellow (including the apex), as can be seen in Figures 1-9.

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Fig. 1. Pine seedlings using chlorella suspension (left) and using distilled water (right).

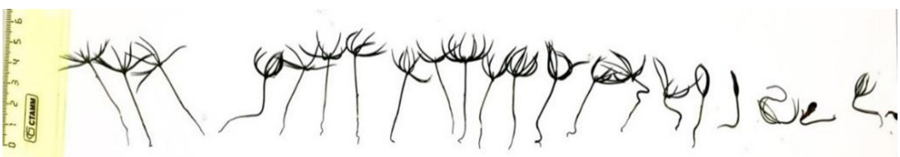

Fig. 2. Spruce seed sprouts using chlorella suspension.

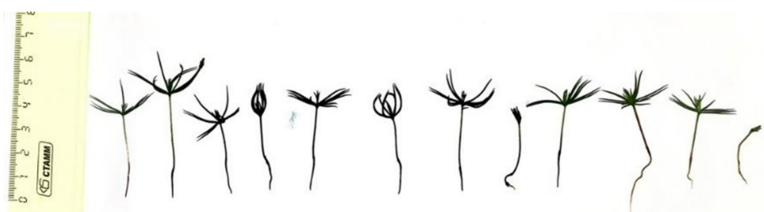

Fig. 3. Spruce seedlings using distilled water (control).

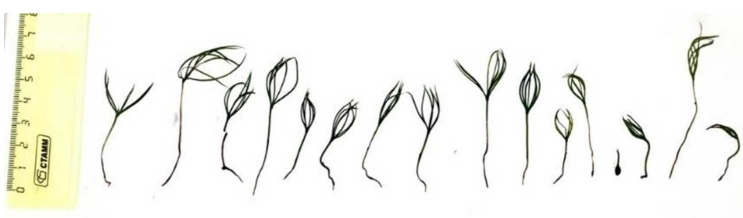

Fig. 4. Pine seed sprouts using chlorella suspension.

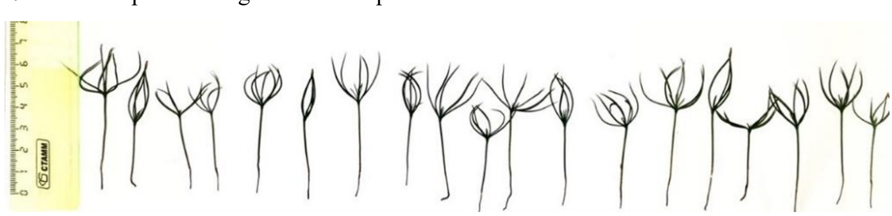

Fig. 5. Pine seedlings using distilled water (control).

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Fig. 6. Marigold seed sprouts using chlorella suspension.

Fig. 7. Seedlings of marigold seeds using distilled water (control).

Fig. 8. Cabbage seed sprouts using chlorella suspension.

Fig. 9. Cabbage seed sprouts using distilled water (control).

The weight of the one triticale plant using chlorella suspension was 0.09 g versus 0.06 g in the control. In addition, we were able to note visual differences: after using the chlorella suspension, the plants had thicker stems, a developed root system, and green leaves, which can be seen in Figure 10.

Fig. 10. Triticale seed sprouts using chlorella suspension (left) and using distilled water (right).

Similarly, the weight of the one rye plant using chlorella suspension was 0.15 g versus 0.06 g in control, and the weight of the one barley plant using chlorella was 0.11 g versus 0.07 g in control. Visual differences in the state of plants are shown in Figures 11-12.

Fig. 11. Rye seed sprouts using chlorella suspension (left) and using distilled water (right).

Fig. 12. Barley seed sprouts using chlorella suspension (left) and using distilled water (right).

In addition, a visual comparison of melissa seedlings allowed us to note the difference in the morphological parameters of the seedlings. When using a suspension of chlorella, melissa leaves turned yellow, in contrast to the control variant, but the mass and volume of the leaves were larger, as can be seen in Figure 13.

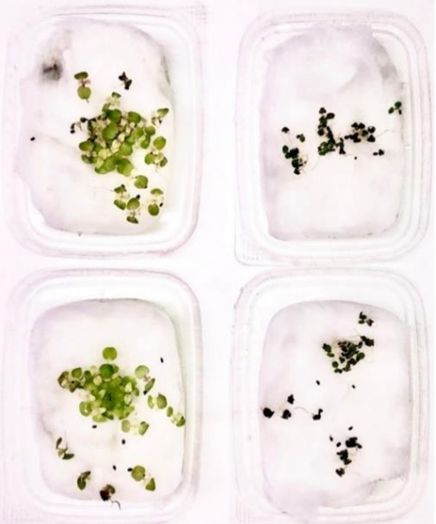

Fig. 13. Melissa seed sprouts using chlorella suspension (left) and using distilled water (right).

4 Discussions

In the last twenty years, there has been an explosive interest in biotechnology based on the cultivation of photoautotrophic microorganisms - cyanobacteria and microalgae - which are a promising source of environmentally friendly and renewable raw materials. Using atmospheric carbon dioxide and sunlight, these objects are able to synthesize a wide range of unique molecules and accumulate valuable bioproducts in their cells: antioxidants, vitamins, polyunsaturated fatty acids, dyes, polysaccharides, UV protectors. In countries with a warm climate, many large enterprises for the cultivation of microalgae have appeared, for example, Cyanotech, Mera Pharmaceuticals Inc. (Hawaii), Jingzhou Natural Astaxanthin Inc (China), Algaetech International (Malaysia), Parry Nutraceuticals (India), etc. In the Russian Federation, there is currently no mass cultivation of microalgae, although the natural and climatic conditions of the Black Sea coast of the Krasnodar Territory, Crimea, Stavropol Territory, are suitable for organizing such production.

At the same time, microalgae and products of their processing are widely used both in animal husbandry and in crop production in a number of countries, and their consumption in this direction is increasing from year to year. One of the most promising areas is their use in agriculture as biofertilizers and biostimulants. Algae extracts contain a large amount of cytokinins, betaine, algopolyphenols, vitamins, hormones, natural antioxidants and mineral elements (Mg, Ca, B, Mo, etc.) that promote plant growth and development and improve soil fertility. The biomass of microalgae is considered as an organic fertilizer, which compares favorably with traditional species, since it contains neither pathogenic microflora, nor weed residues, nor pests. Microalgae are successfully used to improve soil fertility, to replenish organic matter, which helps to increase crop yields.

The biostimulating effect of a suspension of chlorella was demonstrated during the germination of seeds of spruce, pine, marigold, cabbage, rye, barley, spelled, triticale, melissa: the germination of spruce and marigold seeds increased, but the same parameter turned out to be lower compared to the control in pine seeds and the same for cabbage seeds, which demonstrates the difference in the perception of the biostimulator by plants of different taxonomic groups.

Cereals (rye, barley, spelled, triticale) showed a common trend: the mass of plants increased when using a biostimulant based on a suspension of chlorella. Similar results were obtained when applying a suspension of chlorella to the seeds of melissa.

5 Conclusion

Chlorella is an actual object of study due to its wide use in various areas of the national economy, such as agriculture.

It has been shown by various authors that microalgae-based biostimulants can be used as foliar or root application to accelerate plant development or increase overall yield.

The use of a biostimulant based on a suspension of chlorella in the study of seed germination and biometric parameters of seedlings showed similar results: leaf mass and volume, germination, stem and root length increased in plants of spruce, marigold, rye, barley, spelled, triticale, melissa, while in pine plants the germination decreased, and in cabbage plants it remained at the same level, which indicates a difference in the perception of chlorella suspension by plants of different taxonomic groups.

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