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RESEARCH ARTICLE

Effect of *Spirulina platensis* (Gomont) Geitler Extract on Seed Germination of Wheat and Barley

Füsun Akgül*

Mehmet Akif Ersoy University, Faculty of Science and Arts, Department of Molecular Biology and Genetic, Burdur/Turkey

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ABSTRACT

Cyanobacteria has a highly diversity group that consists of photosynthetic prokaryotic microorganisms. Cyanobacteria that produce lots of metabolites such as amino acids, proteins, vitamins etc. have a wide spread. In this study, the effects of different concentrations of *Spirulina platensis* extracts on the germination of wheat and barley seeds and root-stem length, lateral root number and fresh-dry weight were investigated. The application of S5 (100% cell extract) showed an inhibitory effect on seed germination on both wheat and barley. S2 (25% cell extract) and S4 (75% cell extract) applications had a positive effect on germination and seedling development in wheat. In barley, S2 (25% cell extract) application activated germination and seedling growth and other concentration applications did not create a positive effect. As a result; cyanobacterial extract has positive effects on seed germination and plant growth-development and it is possible to produce a commercial and ecological biostimulant by developing different extract concentrations.

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Introduction

The increase in the world population forced the agricultural countries to take more products from the unit area and made it necessary to carry out studies to increase this. However, while carrying out such studies, practices have been carried out giving serious harmful to aquatic and terrestrial ecosystems. The amount of artificial fertilizers using that the most harmful of these applications is increasing day by day, when the unconscious using is added to this process, the ecosystem has irreversible damage. Scientists who take these effects into consideration, have started to work on the production of nature-friendly, biofertilizer-bitostimulant and its use. For this purpose, bacteria, cyanobacteria and algae have been used extensively and effectively.

In the countries such as Norway, Ireland, France and the United States located in the seaside, have been looked for the ways to benefit from the algae and to use them as biofertilizer (Özdemir et. al., 2015). In recent years, numerous studies have been carried out in which cyanobacteria and microalgae have been used as biofertilizer-biostimulants and promising results have been obtained from these studies. Mogor et al. (2017) investigated the effects of *Arthrospira platensis* (*S. platensis*) hydrolysate on the growth of lettuce plants and found that hydrolysates showed cytokine-like effects in lettuce seedlings. Grzesik et al. (2017); researched the foliar applications effects of the biofertilizer that consists of *Microcystis aeruginosa*, *Anabaena* sp., and *Chlorella* sp. species cultures on willow and they detected that increased the plant growth and decreased the amount of artificial fertilizers needing. Muñoz-Rojas et al. (2018) who did the restoration studies with local plant species

* Corresponding author
E-mail address: fakgul@mehmetakif.edu.tr

of Australia, they covered the seeds of these plants with cyanobacteria and then planted them in the soil. They noted a significant increase in the germination percentage of seeds compared to those not covered with cyanobacteria.

S. platensis is a member of a filamentous cyanobacteria and can be used as a food additive in terms of its high protein content and nutritive properties. *Spirulina* sp. has a natural distribution in alkaline environment generally, which prevents its easy contamination (Olguín et al., 1997). In this way, it becomes a suitable organism for use in ecological studies. In addition, *Spirulina* sp. is a good protein supplement in animal nutrition as well as an alternative to chemical fertilizers (Habib et al., 2008). The fact that approximately 60% of the *S. platensis* biomass is protein allows this biomass to be used to obtain protein hydrolysates containing valuable biomarkers. Decarboxylation of these molecules provides polyamine synthesis and the presence of polyamine enables cyanobacteria to be used effectively in plant growth (Kim et al., 2014; Zhang and Zhang, 2013; Lisboa et al., 2016).

In this study; *S. platensis* was cultured continuously and then biomass was extracted and different concentrations of solutions were prepared. The effects of these extracts on the germination of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) seeds which are the most produced 2 monocotyl species in our country were investigated. Germinated seed number, germination percentage, germination energy, body shoot length, total root length, lateral root number and fresh-dry weight parameters have been determined. It has been investigated whether these extracts have a stimulatory effect on seed germination and it is aimed to produce an alternative organic biostimulant from these extracts.

Materials and Methods

Cultivation and Harvesting

S. platensis, obtained from Mehmet Akif Ersoy University, Algal Biotechnology Laboratory, was cultivated flasks using standard Zarrouk culture medium (Zarrouk, 1966), bubbled with air. The biomass was harvested by centrifugation at day 20 of cultivation. The biomass dried in an oven at 45˚C for 24 hours and then powdered with a grinder and stored at +4˚C.

Cell Extract

Dried biomass was suspended in distilled water (DIW) at a concentration of 150 g L^{-1}. For obtaining the intracellular extracts, the suspension was extracted with a sonicator. The suspension centrifuged at 22˚C, 6000×g for 6 minutes for removing biomass residue. To minimize potential degradation, the resulting extract supernatant was collected in a flask covered with aluminum foil and stored in a cold room at 4 ˚C. Five different concentration solution were prepared with cell extract. S1, Control, 0% extract (10 mL DIW); S2, 25% (2,5 mL extract, 7,5mL DIW); S3, 50% (5 mL extract, 5mL DIW); S4, 75% (7,5 mL extract, 2,5mL DIW); S5, 100% (10 mL extract). The biomass residue was also stored in the cold room for potential future use.

Seed Experiment

All solutions were replicated three times with ten seeds per replicate. The seeds were sterilized with 10 mL of 5 % solution of sodium hypochlorite for 10 min, rinsed twice with DIW, transferred to sterile Petri plates, and soaked in 10 mL of the S1, S2, S3, S4, S5 solutions for 24 h. Following the 24-h soaking period, the seeds were placed between two 42.5-mm Whatman no. 1 filter papers and allowed to dry for 24 h at room temperature (21 ˚C). Then, the seeds were transferred to a sterile 100-mm Petri plate containing a moist 75-mm Whatman no. 1 filter, which was soaked with 3 mL of DIW. The plates were incubated at 21 ˚C under a 16-h light/8-h dark cycle. Seed germination was checked at 24-h intervals for 10 days and counted as germinated if at least 2 mm of the radicle had emerged. The filter paper for all treatments was saturated as needed with 3 mL of DIW to maintain moisture. Root, shoot, and leaf lengths (mm) were measured with a caliper. Total root length consists of main and lateral roots and total shoot length consists of main and lateral branches. And also number of lateral roots measured and germination percentage (GP), and germination energy (GE) were calculated.

Germination percentage (GP) was calculated as

GP= (number of germinated seeds/total number of seeds) ×100

Germination energy (GE) was calculated according to Hernández Herrera et al. (2013),

GE= (number of germinating seeds on X. day/number of total seeds) ×100

In this research GE of 3., 5. and 7. days were calculated.

Results and Discussion

When the germination percentage graph of wheat seeds examined (Figure 1); It is clear that S2 and S4 applications have a positive effect on seed germination. S2 and S4 applications have 93% germination percentage, this value is 13% more than control group. The application of S5 with 100% extract was able to produce a germination percentage of 23% by creating a germination inhibitory effect.

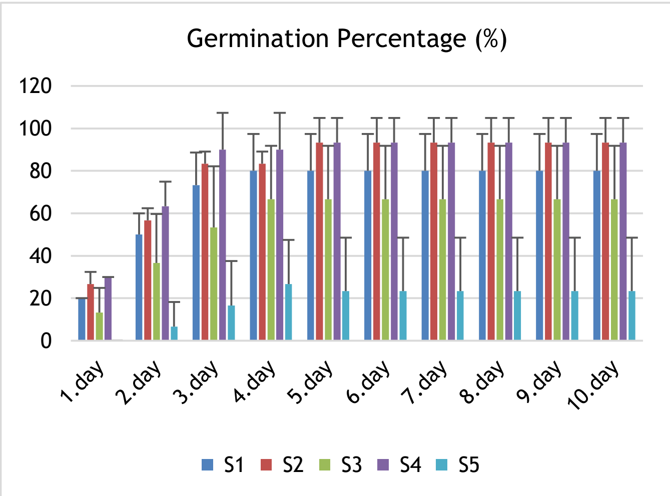

Figure 1. Germination percentage of wheat seeds according to the applications and days

Germination energy is a value indicating the germination rate and it is generally evaluated according to the germination rate of the seeds on the 3rd, 5th and 7th days. It is seen that S4 application in wheat seeds rapidly reaches 90% germination rate on day 3, and S2 application has achieved a rate of 83%. In S2 and S4 applications, a germination energy ratio of 93% is seen on day 5 (Figure 2). In other concentration applications, such a high rate could not be achieved.

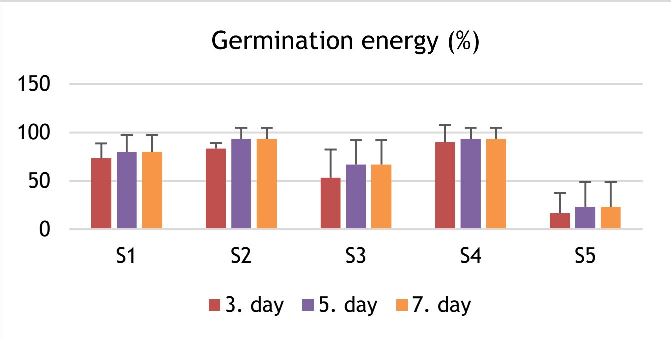

Figure 2. Germination energies of wheat seeds on the 3th, 5th and 7th days according to the applications

When germination percentage and germination rate data are examined, it is seen that S2 and S4 applications have activator effect on germination of wheat seeds.

The graph of wheat total root length (Figure 3) shows that S2 application has more extension, approximately 100 cm according to control group. The S5 application seems to slow down root growth, but S4 seems to be effective even if it is not as effective as S2 in root growth.

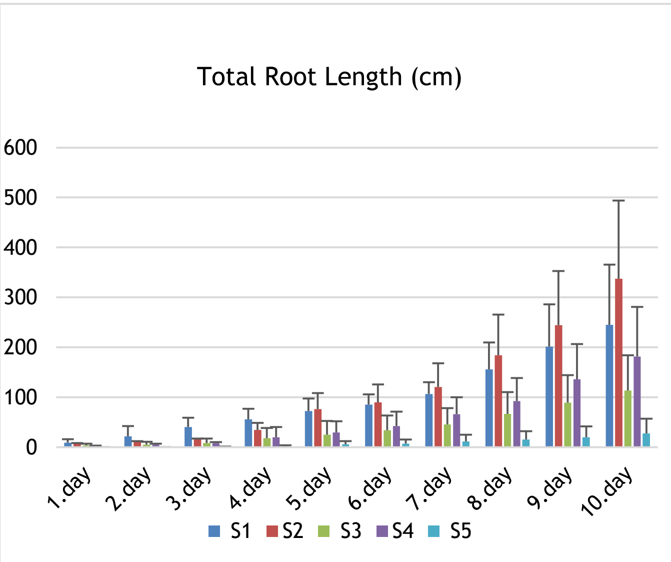

Figure 3. Total root length of wheat seedlings according to the applications and days (cm)

It is seen that the effects of the applications in the body shoot length are similar to the root length effects. The S2 application provided an extension of about 15 cm more than the control group. Again, S4 application almost extended the S1 (control) application (Figure 4). However, S5 application had an inhibitory effect on shoot extension.

When the lateral root number graph was examined (Figure 5), S2 and S4 applications were caused to more lateral root formation than the control group. And this result shows that this extracts have a stimulating effect on plant growth.

Dry weight data revealed that S2 and S4 applications had positive effects (Figure 6).

When all these data are examined; it is seen that S2 (25%) and S4 (75%) applications have a stimulating effect on germination and seedling development of wheat germ. Much better germination and development results than the S1 (control) group shows that *S. platensis* extract can be used as a biostimulant in seed germination.

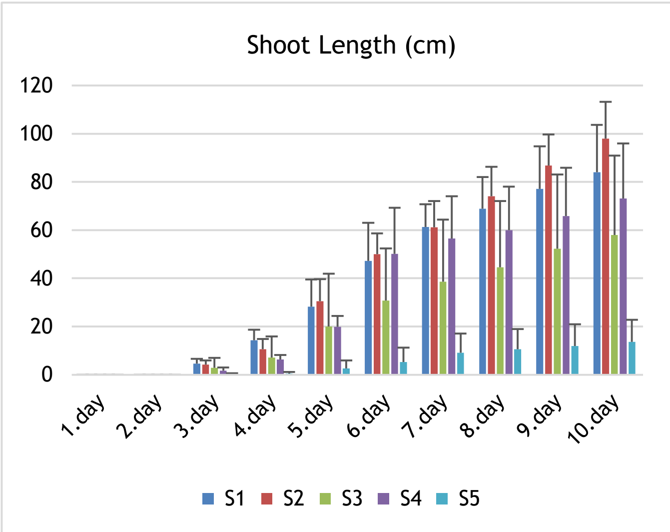

Figure 4. Shoot length of wheat seedlings according to the applications and days (cm)

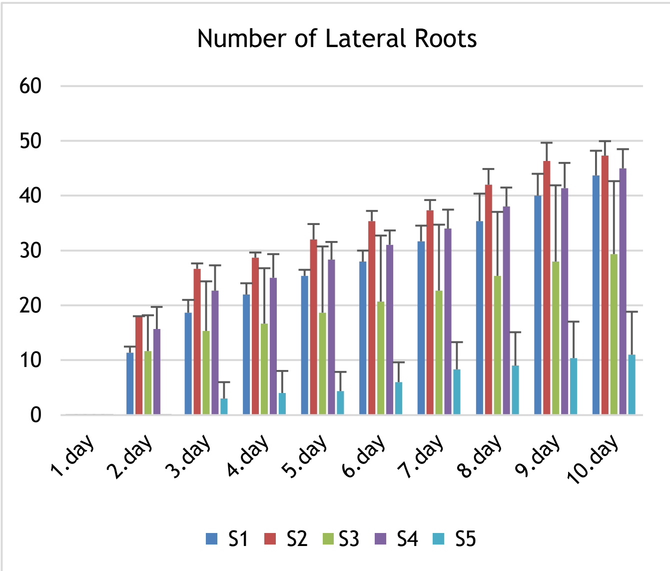

Figure 5. Number of lateral roots of wheat seedlings according to the applications and days (piece)

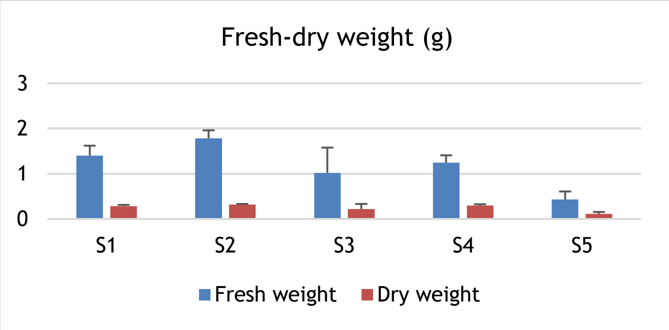

Figure 6. Fresh and dry weights of wheat seedlings according to applications (g)

Similar to this result; Michalak et al. (2016); investigated the effects of some algae and *S. platensis* extracts on wheat development, especially in the *S. platensis* extract group, the number of grains in the wheat ear and the length of the stem were significantly higher than the others. In addition; Khushwaha and Banerjee (2015); the effect of the addition of cyanobacterial culture on chickpea, rice and wheat seed germination on the low temperature, in the laboratory conditions were investigated and the germination percentages, root-stem length, number of leaves, chlorophyll amount found more than the control group that without cyanobacteria culture. Shariatmadari et al. (2015); have applied the extracts of different cyanobacterial members to wheat seeds and they noticed that germination percentage does not change much, but the seed and root length of the seedlings more extended than the control group. In another study, Gahlout et al. (2017); researched that the effects of the extracts of different cyanobacteria on wheat and mung bean seed germination and seedling development in their studies and they detected that extracts increased the percentage of seed germination (at the end of the 3rd day, more germination percentage -70% excess-was observed than the control group) and extract has a positive effect on seedling development. We found similar results in Kumar and Kaur (2014); *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima* and *Tolypothrix tenuis* filtrates applied to wheat seeds, and they found a positive effect on seedling growth compared to those without filtrate.

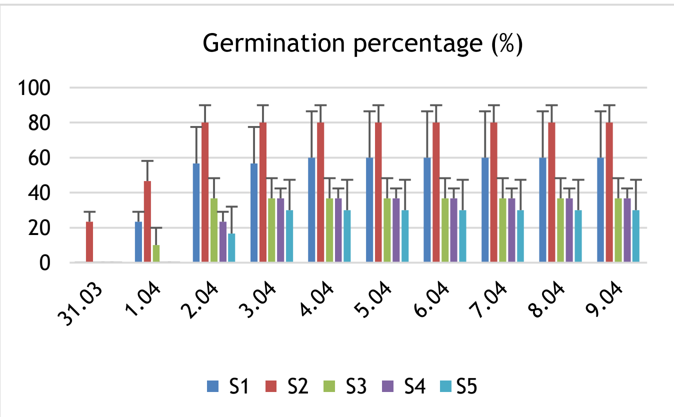

Figure 7. Germination percentage of barley seeds according to the applications and days (%)

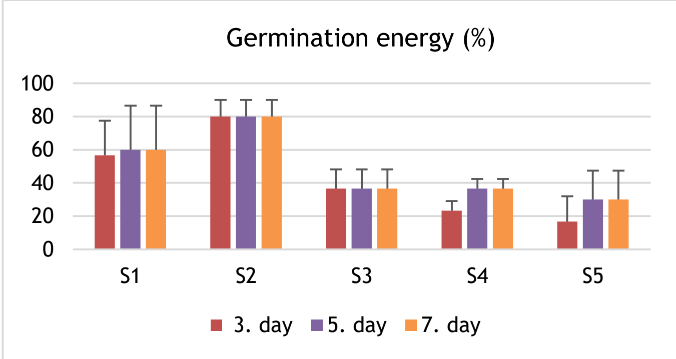

Figure 8. Germination energies of barley seeds on the 3th, 5th and 7th days according to the applications (%)

When the germination percentage and energy of barley seeds are examined (Figure 7 and 8); It is seen that S2 application has a positive effect on germination of barley seeds and accelerates germination.

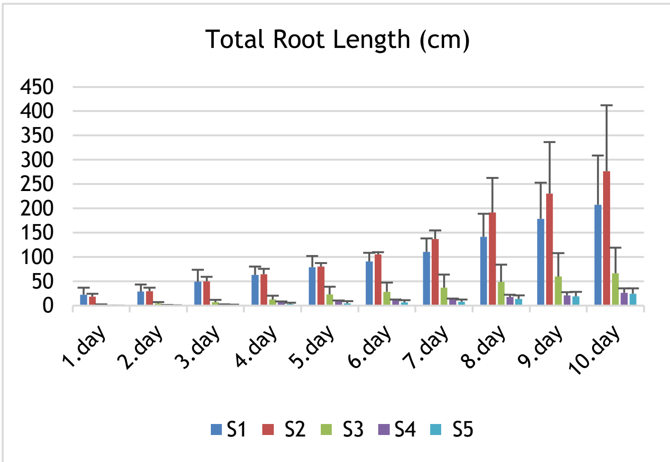

Figure 9. Total root length of barley seedlings according to the applications and days (cm)

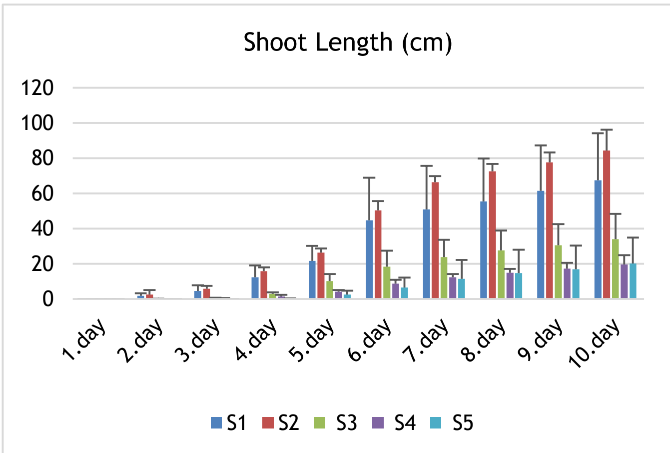

Figure 10. Shoot length of barley seedlings according to the applications and days (cm)

Total root length and shoot length graphs of barley seedlings were examined (Figure 9 and Figure 10); S2 application has a positive effect on root and shoot development and compared to S1 (control) application;

approximately 70 cm in root length, 17 cm in body length more elongation were observed.

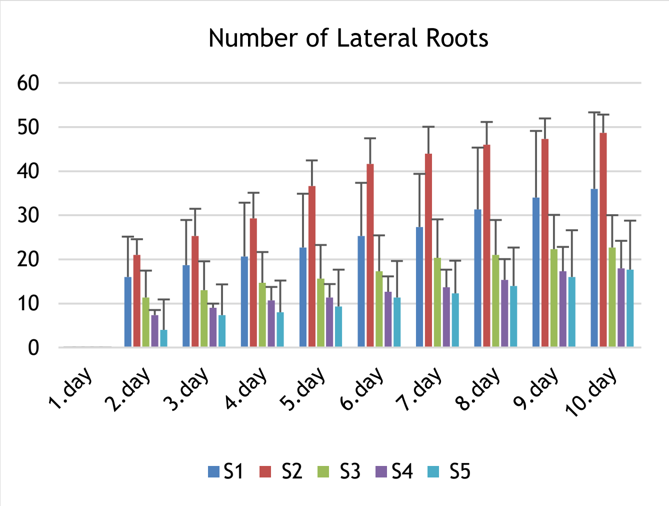

Figure 11. Number of lateral roots of barley seedlings according to the applications and days (piece)

Figure 11 shows that S2 application significantly increases the number of lateral roots in barley compared to S1 (control) application and it affects the seedling development positively. The total number of lateral roots in the seedlings at the end of the 10-day; was 36 in the S1 (control) application and 48.6 in the S2 application.

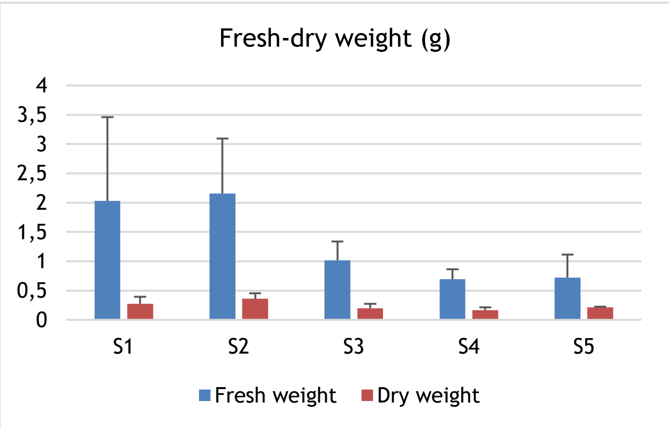

Figure 12. Fresh and dry weights of barley seedlings according to applications (g)

At the end of 10 days, the fresh and dry weight graph of barley seedlings (Figure 12) also showed that S2 application had a positive effect on biomass increase.

When all the data related to barley seeds are evaluated; it is observed that S2 (25%) application has positive effects on seed germination and seedling development. It can be said that *S. platensis* extract accelerates the seed germination and have biostimulant effect in plant growth. S4 and S5 applications showed a slowing effect as close to each other compared to S1 (control) application. The use of intensive extracts has an inhibitory effect but the extracts in certain doses activate the growth.

Ismail and Hamad (2017); used *Anabaena variabilis* extracts in their work and stated that this extract increases the germination percentage, root-shoot length and fresh-dry weight of the seeds of *Hordeum vulgare* and *Trigonella foenum-graecum* L. Shariatmadari et al. (2013); used the extracts of *Anabaena* and *Nostoc* genus in the development of rice plants and stated that they affected plant growth positively. Essa et al. (2015); investigated the effects of some cyanobacterial exudates on sorghum and sunflower seed germination and seedling development and found that *Anabaena oryzae* has been very effective on seed germination and root-shoot development. Grzesik and Romanowska-Duda (2014); applied *Microcystis aeruginosa*, *Anabaena* sp. (Cyanobacteria), and *Chlorella* sp. cultures to commercial corn plants; and detected positive effects on seed germination and seedling growth. Win et al. (2018); stated in their review study that algal biofertilizers should be used strictly for sustainable agriculture.

Conclusion

All these data indicate that the bio-fertilizers containing cyanobacterium extracts that are natural and dissolve in nature spontaneously should be preferred instead of artificial fertilizers which cause bioaccumulation in living systems, because of their positive effects on plant growth and non-destructive properties.

Although there are many benefits of this organic biofertilizer, some obstacles in the process of commercialization. These obstacles can be overcome by explaining the biofertilizer to the farmer very well. In the future studies, the chemical compositions of microalgae and cyanobacterial extracts should be characterized in detail and the most effective concentration and composition should be developed and this product should be placed in the fertilizer sector by emphasizing the ecological and economic importance of this product. Besides; similar trials should be carried out in the field and the results should be discussed carefully.

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References

Essa A.M.M., Ibrahim W.M., Mahmud R.M., El Kassim N.A., 2015. Potential impact of cyanobacterial exudates on seed germination and antioxidant enzymes of crop plant seedlings. *International Journal of Current Microbiology and Applied Sciences*,4 (6):1010-1024.

Gahlout M., Prajapati H., Chauahan P., Himitakumari R. ve Patel J., 2017. Isolation, Identification and Evaluation of Seed Germination Efficiency of Cyanobacterial Isolates International Journal of Research and Scientific Innovation (IJRSI)Volume IV, Issue IV.

Grzesik M., Romanowska-Duda Z., 2014. Improvements in Germination, Growth and Metabolic Activity of Corn Seedlings by Grain Conditioning and Root Application with Cyanobacteria and Microalgae. *Pol. J. Environ. Stud.* 23(4):1147-1153.

Grzesık M., Romanowska-Duda Z., Kalajı H.M., 2017. Effectiveness of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (*Salix viminalis* L.) plants under limited synthetic fertilizers application. Photosynthetıca 55 (3): 510-521, doı: 10.1007/s11099-017-0716-1.

Habib M.A.B., Parvin M., Huntington T.C., Hasan M.R., 2008. A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish, FAO Fish. Aquac. Circ. (2008) (No. 1034).

Hernandez-Herrera R.M., Santacruz-Ruvalcaba F., Ruiz-López M.A., Norrie J., Hernández-Carmona G., 2013. Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). J Appl Phycol 26:619–628.

Ismail G.A. ve Abo-Hamad S.A., 2017. Effect of Different *Anabaena variabilis* (Kütz) Treatments on Some Growth Parameters and Physiological Aspects of *Hordeum vulgare* L. and *Trigonella foenum-graecum* L. *Egypt. J. Bot.,* 57(3):507–516.

Khushwaha M., Banerjee M., 2015. A Novel Method of Seed Germination and Growth of Three Staple Crop Plants: Effect of Low Temperature and Cyanobacterial Culture Addition. J. Algal Biomass Utln. 2015, 6 (1): 26-32.

Kim N.H., Jung S.H., Kim S.H., Ahn H.J., Ong K.B., 2014. Purification of an iron-chelating peptide fromSpirulina protein hydrolysates. J Korean Soc Appl Biol Chem 57:91–95.

Kumar A. ve Kaur R. 2014. Impact of Cyanobacterial Filtrate on Seed Germination Behaviour of Wheat. International Journal of Basic and Applied Biology, 1(1):11-15.

Lisboa C.R., Pereira A.M., Costa J.A.V., 2016. Biopeptides with antioxidant activity extracted from the biomass of *Spirulina* sp. LEB 18. Afr J. Microbiol Res 10:79–86.

Michalak I., Chojnacka K., Dmytryk A., Wilk R., Gramza M., Rój E. 2016. Evaluation of Supercritical Extracts of Algae as Biostimulants of Plant Growth in Field Trials. Front. Plant Sci. 7:1591. doi: 10.3389/fpls.2016.01591.

Mógor Á.F., Ördög V., Lima G.P.P., Molnár Z. ve Mógor G., 2017. Biostimulant properties of cyanobacterial hydrolysate related to polyamines. J Appl Phycol., DOI 10.1007/s10811-017-1242-z.

Muñoz-Rojas M., Chilton A., Liyanage G.S., Erickson T.E., Merritt D.J., Neilan B.A., Ooi M.K.J., 2018. Effects of indigenous soil cyanobacteria on seed germination and seedling growth of arid species used in restoration. Plant Soil 429:91–100, https://doi.org/10.1007/s11104-018-3607-8.

Olguín E.J., Galicia S., Camacho R., Mercado G., Peŕez T., 1997. Production of *Spirulina* sp. in sea water supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. Appl. Microbiol. Biotechnol. 48:242–247.

Özdemir S., Sukatar A. ve Öztekin G.B., 2015. *Chlorella vulgaris* Üretimi ve Sera Organik Domates Yetiştiriciliğinde Biyogübre Olarak Kullanımının Etkileri. Tarım Bilimleri Dergisi, 22:596-605.

Shariatmadari Z., Riahi H., Abdi M., Hashtroudi M.S. ve Ghassempour A.R., 2015. Impact of cyanobacterial extracts on the growth and oil content of the medicinal plant *Mentha piperita* L. J Appl Phycol, DOI 10.1007/s10811-014-0512-2.

Sharıatmadarı Z., Rıahı H., Seyed Hashtroudı M., Ghassempour A.R. ve Aghasharıatmadary Z., 2013. Plant growth promoting cyanobacteria and their distribution in terrestrial habitats of Iran. Soil Science and Plant Nutrition, 59:535–547.

Win T.T., Barone G.D., Secundo F. ve Fu P., 2018. Algal Biofertilizers and Plant Growth Stimulants for Sustainable Agriculture. Industrıal Bıotechnology, doı: 10.1089/ind.2018.0010.

Zhang B., Zhang X., 2013. Separation and nanoencapsulation of antitumor polypeptide from *Arthrospira platensis*. Biotechnol Prog 29:1230–1238.