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THE EFFECT OF SPIRULINA PLATENSIS (GOMONT) GEITLER EXTRACTS ON SEED GERMINATION OF LACTUCA SATIVA L.

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Abstract

Because of increasing human population and their food need the agricultural production increases, too. But meanwhile artificial fertilizer using and their negative effects to ecosystem are rising day by day. So, the scientist research different alternative solution for this pollution such as eco-friendly biofertilizer.Group of Cyanobacteria consists of photosynthetic prokaryotic microorganisms that has a highly diversity. Cyanobacteria can produce different metabolites that are valuable economically such as amino acids, proteins, vitamins etc. This study focuses the effects of different concentrations of *Spirulina platensis* (Gomont) Geitler extracts on the germination of lettuce seeds. For this purpose, root-stem length, lateral root number and wet-dry weight were investigated. The application of S5 (100% cell extract) showed an inhibitory effect on seed germination and so other parameters could not be measured. S2 (25% cell extract) and S3 (50% cell extract) applications had a positive effect on germination and seedling development in lettuce. 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. And this biostimulant may be used instead of the other ecologically harmful artificial fertilizer. By the way; the large amount money that spends for the artificial fertilizer will be brought to the economy of Turkey.
Keywords: Biostimulant, Cyanobacteria, Lettuce, Seed Germination, *Spirulina Platensis*.

1. Introduction

The human population is increasing day to day all over the world and their nutrient requirement, too. However, the quality and quantity of farming areas, water resources and agricultural products decrease. So, the farmers have to obtain more crop per farming area without any pollution that because of artificial fertilizer. And for this aim scientist started to research biofertilizer and biostimulant that affect plant growth positively.

In recent years, photosynthetic microorganisms draw attention to benefits in many sectors such as wastewater treatment, animal feeds, biodiesel, bioethanol, biofuel production, fertilizer and nutrient production (Win et al., 2018).Cyanobacteria are the simplest living autotroph microorganisms that produce huge amount of oxygen. And also they produce organic material from inorganic compounds and when they stressed, they produce seconder metabolites are very useful for the plant growth (Malliga et al., 2002; Kheirfarm et al., 2017).They have a wide distribution in the ecosystem and tolerance to ecological stress factors (de Marsac and Houmard, 1993).

For these characteristics of cyanobacteria; effects of *S. platensis* extract on seed germination of lettucewere investigated in this research. And the optimal concentration of extract was detected for seedlings for the purpose of creating possible commercial bio stimulant instead of artificial ones.

2. Material and Methods

2.1 Cultivation and Harvesting

S. platensis, obtained from Mehmet AkifErsoy University, Algal Biotechnology Laboratory, was cultivated flasks using standard Zarroukculture 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.

2.2 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.

2.3 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. 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 HernándezHerrera 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.

2.4 Statistical Analysis

Each application concentration was analyzed on three biological replicates. The reported values are the means ± standard deviation of three values. Data were analyzed using two-way analysis of variance (ANOVA) using Microsoft Office Excel 2007. A significant difference was considered at level of $p < 0,0001$.

3. Results and Discussion

It is seen clearly in all figures;S5 treatment has negative effect on seed germination and so the other parameters could not be measured. Similarly, in our results Sornchai et al. (2014) researched effects of *S. maxima* extract on different plants and resulted their research that there is no beneficial effect to seed germination and they showed different reactions on plant growth according to extraction solvent.

In the early days of observation; S3 treatment is effective for accelerating seed germination, but in 3th and later days, this treatment has same effect with control treatment. In general, S1, S2, S3 treatment have similar effect on seed germination, but S4 blocked the germination (Figure 1 and 2). Similarly, in seed germination; S4 treatment has no significant impact on root length. However, S2 treatment has more positive effect on root elongation than control treatment. And also S3 has effect almost as same as control (Figure 3).In shoot length (Figure 4); S2 treatment has the most important effect, then S1, S3 and S4, respectively. Control treatment (S1) has the foremost effect in lateral root number, it means that other treatments are not necessary for rooting (Figure 5). S2 create more fresh and dried weight than S1 and also other treatments (Figure 6).

Aly and Mona (2008) recorded that *S. platensis* is a suitable bio stimulator for pepper plants. Wuang and et al. (2016) indicated that *Spirulina*-based fertilizers enhance plant growth such as leafy vegetables.Alves Dias et al. (2017) indicated that *S. platensis* fertilizer (Spirufert) play important role to plant growth and vegetable crop. Brahmbhatt and Kalasariya (2015) used *Spirogyra* and *Oscillatoria* cyanobacteria and detected that presoaking seed by extracts of cyanobacteria accelerates seed germination and also *Spirogyra* more effective on plant growth than *Oscillatoria*. Mohsen, et al. (2016) indicated that two cyanobacterial extracts (*Anabaena oryzae* and *Nostocmuscorum*) for lettuce plants significantly increased the plant height, number of leaves /plant, head weight and total yield of lettuce plants compared with control treatment.

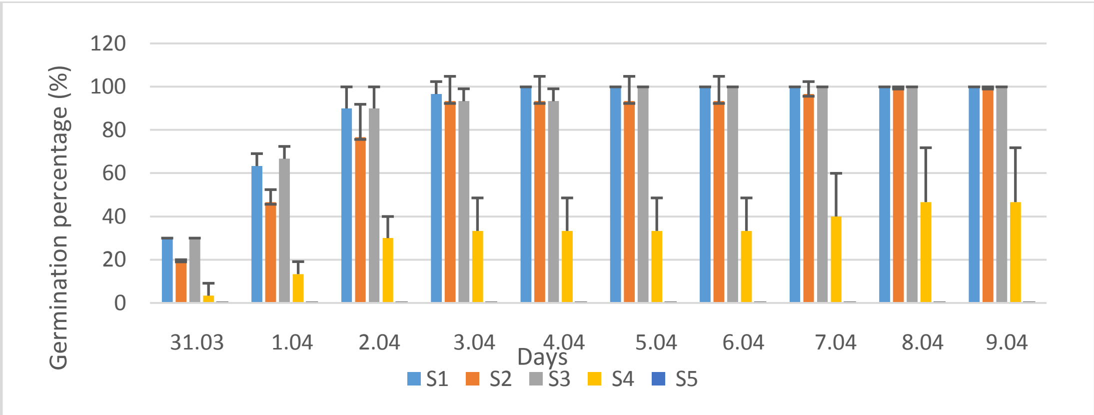

Figure 1. Germination Percentage of Lettuce Seeds According to the Applications and Days (%).

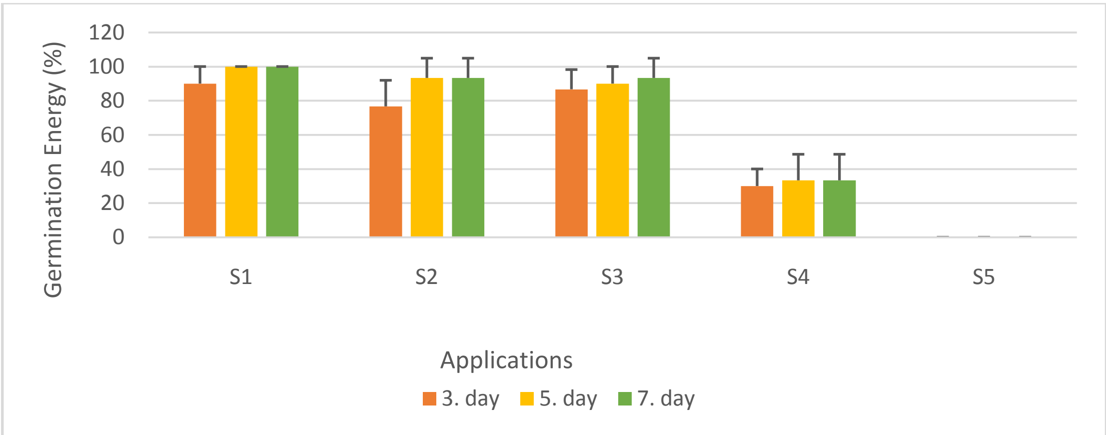

Figure 2. Germination Energies of Lettuce Seeds on the 3th, 5th and 7thDays According to the Applications (%)

Unlike the above studies; Bhowmik et al. (2011); detected in their study that used *Spirulina* as inoculant for Pulses; different reactions observed for plant growth and inoculation of *Spirulina* shows no positive effects in the crop plants.

All these results show that *S. platensis* extract is highly effective for lettuce seedlings. And this or other concentrations that would be adjusted according to this study may be used instead of artificial fertilizers. In future works need to be done to develop new bio efficacy concentrations and maintain under field environment conditions. It is so important for the sustainable environment, because synthetic fertilizers have long-term negative effects. They kill benefit microorganisms, change pH in the soil, pollute groundwater and increase its toxicity and also all aquatic system and the final all ecosystem. In this respect; the studies that aim to create eco-friendly, organic and bio degradable fertilizer like this study are very valuable for the natural life because of affecting all living things.

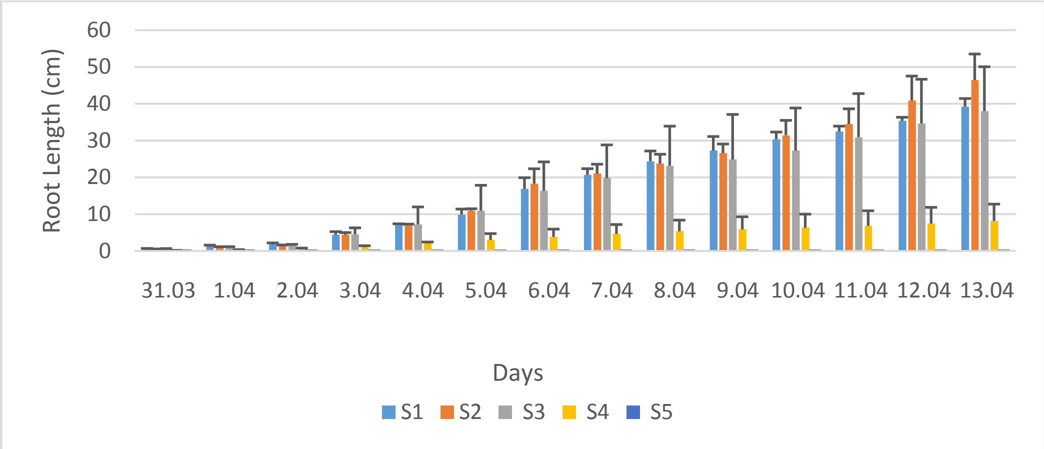

Figure 3. The Root Length of Lettuce Seedlings According to the Applications and Days (cm)

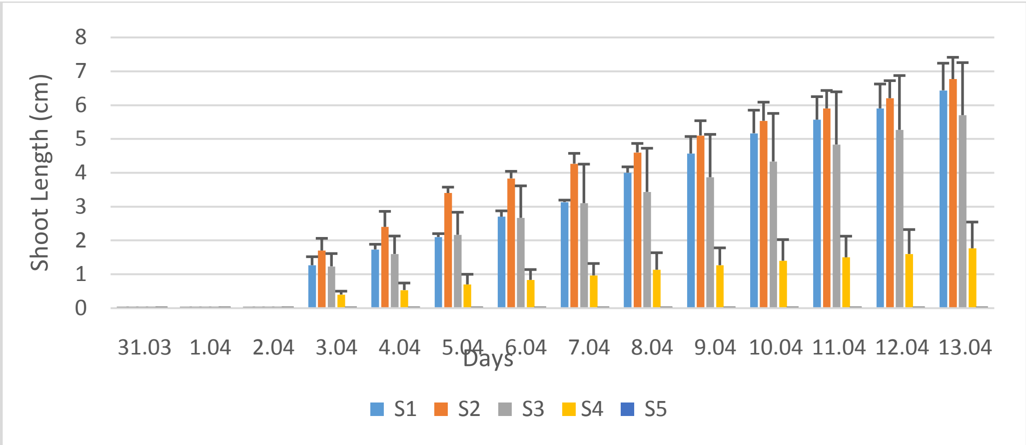

Figure 4.The Shoot Length of Lettuce Seedlings According to the Applications and Days (cm)

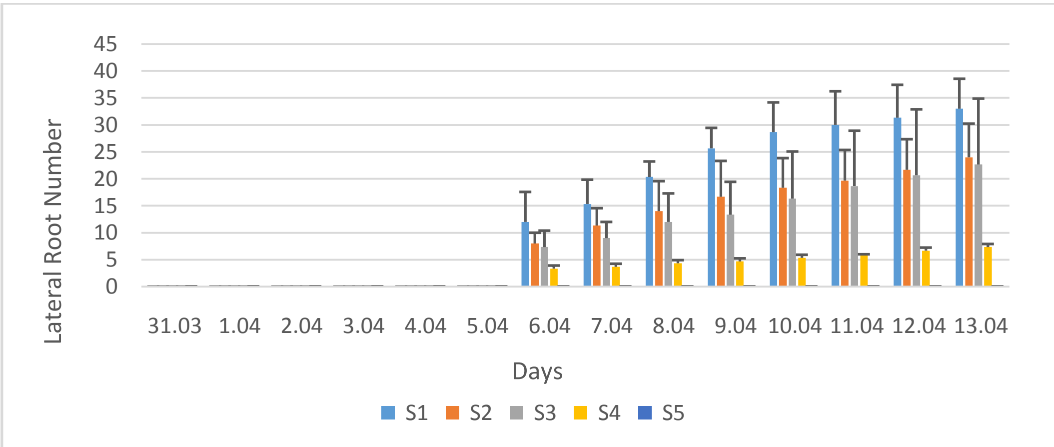

Figure 5.Number of Lateral Roots of Lettuce Seedlings According to the Applications and Days (piece)

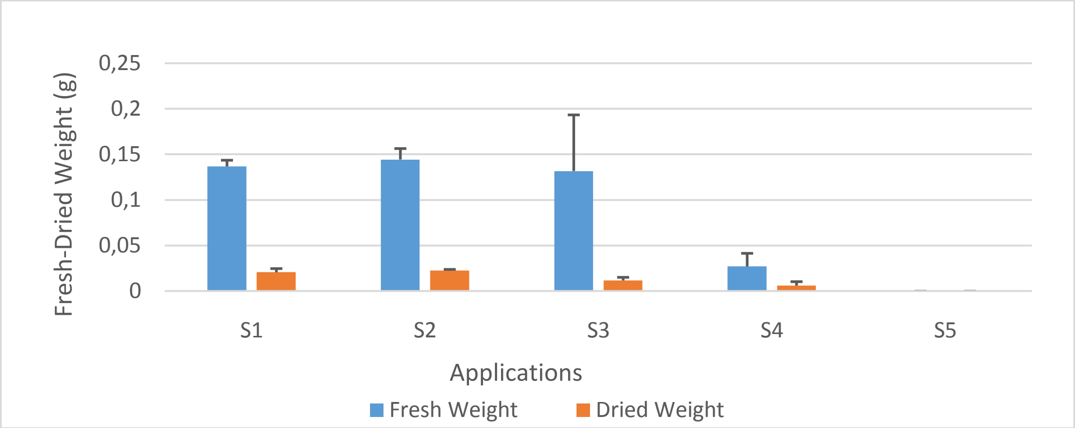

Figure 6.Fresh and Dried Weights of Lettuce Seedlings According to Applications (g).

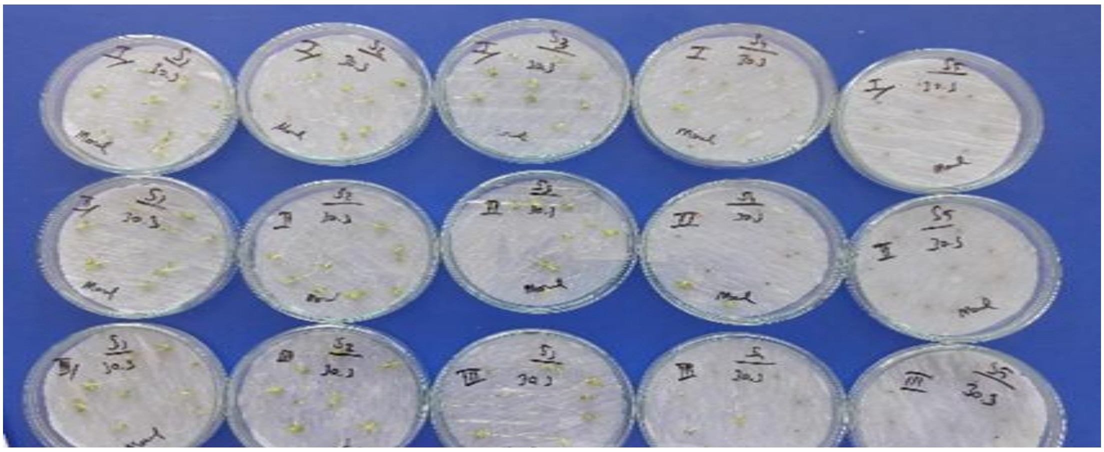

Figure 7. Lettuce Seeds and Seedling Development on the Last Day of İncubation

4. Conclusion

Artificial fertilizer causes ecological damage and creates serious economic expense. Creating and producing a bio degradable and organic bio fertilizer instead of these artificial fertilizer is very important because of protecting the environment and saving money. Though chemical fertilizers increase crop production; their overuse has hardened the soil, decreased fertility, strengthened pesticides, polluted air and water, and released greenhouse gases, thereby bringing hazards to human health and environment as well. When considering disadvantages and cost of chemical fertilizer and the results of this study; for the sustainable environment and economy according to this and similar studies optimum concentration should be found and mass production should be started commercially.

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